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We are confident that Bulgarian Journal of Soil Science will unite the research of specialists and scientists in soil science from all over the world, as well as help them in the processes of their scientific integration. Bulgarian Journal of Soil Science is an international open-access, web-based scientific journal dedicated to the publication and discussion of high-quality research in the field of soil sciences. Bulgarian Journal of Soil Science publishes scientific research that contributes to the understanding of soil and its interaction with humans and the entire Earth system. The scope of the journal includes all topics relevant to the study of soil science as a discipline. The journal publishes original papers in specific topic sections on global and regional, theoretical and experimental studies concerning the problems of genesis, geography, physics, chemistry, biology, fertility, management, conservation, and remediation of soils.

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To the Readers

Editor-in-Chief

Prof. Metodi Teoharov

Dear readers of Bulgarian Journal of Soil Science,

Dear colleagues,

On the occasion of marking 2015 as the International Year of Soil the Managing Board of Bulgarian Soil Society decided to release its own online journal. After accepting the general publishing requirements and confirming the approved sections of the journal the management of the Editorial Board prepared the first issue. I believe that the journal will be greeted by all readers and colleagues both abroad and in our country as we are all aware that soil is a unique natural resource and it is as valuable as water and air for every living being. We must know and preserve soil because it is a living biological system which is subjected to the impact of external and internal factors of any kind. That is why care of soil is in fact care of people. The modern man rarely remembers that soil is the first born offspring of Mother Earth and it is characterized by the inestimable quality of fertility. Knowledge, conservation and protection from all sorts of abuse is an obligatory prerequisite for ecological and sustainable soil management.

Soil scientists, no matter where in the world they are, are always guided by the Dokuchaev and neo-Dokuchaev paradigm “properties – processes – factors of soil formation” and this is the basis on which they suggest measures for preservation of soil fertility. This proven formula most accurately determines its genesis, evolution and changes in time and space. In certain historical periods this model of natural law was violated and parts of the Earth broke off and formed new lands. Huge land ecosystems, cities and islands remained under water flooded by seas and oceans. Historical artifacts show that soil has preserved the traces of lost civilizations. It possesses the historical memory “to store” climatic changes, rocks, vegetation, animals and people. In the profile of Red Cinnamon Forest Soil on Bulgarian land particles of relics of St. John the Baptist were discovered. Today under the surface of the soil cover explorers find sanctuaries, Thracian tombs, fortress walls, fine mosaics, chariots, amphorae and gold treasures.

Soil is a national century-old fortune, an irreplaceable natural resource, which is used for multiple purposes and in comparison with the other components of the ecosystem it virtually has very specific natural functions. The attitude of man and society to soil is actually attitude to themselves and to the future generations. Soil is a natural and historical body, which creates and reproduces material and spiritual values. It is a complex structurally organized system, which has components similar to those of Man: solid phase (skeleton), gaseous phase (respiratory system), liquid phase (blood system) and living matter (heart pulse). As a complex multifunctional system soil patiently endures the vagaries of time and man. Modern people build huge motorways and seal under asphalt and concrete dearest black earth, they surround themselves with concrete walls and palaces and build housing estates, new cities, petrol stations, shopping malls. They create numerous dumpsites, tailings and embankments, which scarify soil with heavy metal and organic waste. We must not forget that soil is organically linked to the living and non-living natural habitat and if they are separated this fact can play “a bad joke” to the Earth. We must not forget that in such case soil is turning into a non-renewable resource.

All this obliges us to accept soil as both our own and Nature’s creation which is closely linked to the future of many generations. That is why it is necessary to know its genetic essence and to protect it from the impact of all natural forces and Man’s violations. Ignorance of soil as the primary means of creating material and spiritual benefits, its irrational use move people away not only from nature but also from their genetic essence and habitat. That is why the main goal that Bulgarian Journal of Soil sets to itself is being recognized by the Bulgarian and world soil community as their scientific edition so that every research fellow and preservationist can publish their ideas in the journal.

The journal will deal with contemporary topics and tasks, connected with ecology, climatic changes, the origin, diagnostics, classification, use and preservation of soil and land resources as well as increasing their fertility. Therefore, the main sections to which the journal will adhere are as follows: Genesis, Geography and Classification of Soils, Soil Physics and Agrophysics, Soil Erosion and Degradation, Agrochemistry and Agroecology, Agrotechnics and Hydromelioration, Soil Microbiology, Biodiversity, Biosphere, Technosphere and Noosphere, Plant Protection, Ecology and Preservation of Soil Resources and Environment.

I believe that the journal will provide answers to a number of questions raised by modern soil science. With our first issue the Science Editorial Board and all Bulgarian soil scientists appeal to all people on our planet: “Preserve the soil and soil fertility as this is the eternal and inestimable treasure for our children and for all of us!”

Good luck to Bulgarian Journal of Soil Science!
Founders of the Journal:
Prof. Metodi Teoharov, Asst. Prof. Biser Hristov, Assoc. Prof. Ludmila Malinova, Assoc. Prof. Radka Donkova, Assoc. Prof. Rada Popova, Assoc. Prof. Toma Shishkov and Asst. Prof. Katerina Doneva

Editor –in-Chief: Prof. Metodi Teoharov, PhD
President of Bulgarian Soil Science Society

One Hundred and Five Years of Soil Science in Bulgaria

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Abstract

In this article is presented stage development of Bulgarian Soil Science – from 1911 year until our days. Fonder of soil science of Bulgaria is Nikola Pushkarov. Nearly, with his life is finished the first stage (1947). Pushkarov developed the first scientific program of soils with basic tasks.

Second stage of development (1947-1969) is stage of rapid development of soil science associated with the reorganization of agriculture in Bulgaria.

Third stage (1969-1989) is stage of modernization of equipment, strengthening of international relations and recognition, advanced science servicing of agriculture.

Fourth stage is stage of reforms, changes and integration into European research area.

Keys words: Nicola Pushkarov, Bulgarian Soil Science, stage development, science programs, soil diagnostic and classification, soil melioration, research, agroecology and agriculture, reform, changes and integration.

Creative thought of the greatest scientists has persistently strived to reveal the secrets of nature associated with soil and its fertility, to develop useful methods for research, increase, rational use and protection of land wealth. Soil is a unique ecological resource for every living creature as well as air and water.

Concern of soil health is as important as concern of human health. Soil science entered the cycle of scientific disciplines serving practice when in 1883 the famous Russian scientist V.V. Dokuchaev established regularities of soil formation.

I. N. Pushkarov - Founder of Soil science. First Stage of Development (1911-1947).

The basics of soil science in Bulgaria was set in 1911 by the talented Bulgarian scientist, naturalist, founder of soil science and agricultural science, great patriot, public and popular educator Nikola Pushkarov. In his earlier years he was the founder and leader of the company of naturalists and students involved in student fights, teacher and organizer of the teacher movement in different parts of the country, lecturer and professor of cultural and educational organizations, environmentalist and afforestation specialist, head of a revolutionary group, participant in the Ilinden uprising in 1903, Chairman of the Skopje Revolutionary Committee, publisher and editor of newspapers and magazines. Pushkarov belongs to that generation of Bulgarians, who grew up after the liberation of Bulgaria and whose national and civic consciousness was formed under the influence of patriotism, the ideals of freedom, independence, social justice and revival of the fighters for national liberation. Although he was an excellent student of natural science and geology, having an invitation and opportunity to work in the Ministry of Education and a popular teacher and organizer of young adults in their fight for more education, culture, economic growth and social justice, Pushkarov did not hesitate to lift the rifle with Gotse Delchev and come to the rescue against foreign oppressors in Macedonia (Raikov, 2001). After the revolutionary struggles he dedicated himself to teaching and social work. He extended his journalistic activities, particularly in the magazine "Natural Science and Geography”, to which the most important representatives of Bulgarian science at that time contributed. Pushkarov is the first editor of the Magazine of Agricultural Research Institutes in Bulgaria, the first translator of the Origin of Species by Charles Darwin in the country, etc. (Kolarova, Dimcheva, 1959, Raykov, 2001; Teoharov, 1981, 2011). After specializing in Germany, Russia and America, he began an extensive soil survey in the Agricultural Experimental Station - Sofia. In 1911 the highly skilled and knowledgeable professional and young scholar founded the Soil Science and Agro-geological department and introduced the scientific principles of Dokuchaev Genetic Soil Science. As head of the department Pushkarov developed the first scientific program including the following tasks:

1. Systematic study of soils in areas by collection of geological, biological and climatic data and analyzing the collected samples in chemical and physical terms.
2. Tracing the causes of changes in soil properties in a given area and an indication of possibilities of eliminating the hazard.
3. Producing maps for the areas of research.

These common programming tasks are as relevant today as they were at the beginning of the 20-th century. As director of the Central Experimental Station, which he transformed into the Central Research Institute in 1920, Pushkarov founded 8 departments. He significantly broadened the Soil Department, where he trained and prepared the first soil scientists in the country and under his management the first regional soil surveys were carried out. In 1913 he published the Soil and Geological Outline of the Sofia Field and published the first regional soil map in 1:120000 Scale. In 1931, with funds borrowed from a bank mortgage on his home which he paid for a lifetime, he published the first national soil map of Bulgarian and German in 1:500000 Scale. This example of selflessness and devotion to science goes far beyond conventional standards (Raikov, 2001). However, he described and classified soils by genetic diagnostic criterion and established the first National Soil Classification. All these studies place under his direction the earliest scientific service of agriculture, offering concrete methods and choices in the field of assessment, treatment and agricultural technique of soil, its degradation, acidification, salinisation, land reclamation, mineral and organic fertilization, the fight against drought, grain production , forage production, tobacco and others. Despite his major contributions to soil science and agriculture Pushkarov was fired unfairly by the current agriculture minister Prof. Yanaki Mollov. Having retired early, Pushkarov began work on the Second National Soil Map with his own funds only to finish his life in poverty and misery. His scientific works, his scientific, organizational and applied research were appreciated four or five years after his death, when he received international and national acclaim, and his name was chosen for the patron of our Institute. His activity was appreciated and he was rightly called Patriarch of the Bulgarian Soil by future generations (Teoharov, 1981).

In the first period of development of soil science, active research began to develop in the Department of General Agriculture and Soil Science in 1924, led by Academician Ivan Stranski in the Agronomy and Forestry Faculty of St. Kliment Ohridski University. In 1929 he wrote the first textbook of Soil Science (253 pages), which was published with private funds. In 1935 the book was released as the first edition of the University, greatly expanded and improved technically too, and designed to serve not only as a textbook but as a guide for agronomists and foresters in their practical activity. The coursebook of soil science, which was released in 1946, was significantly upgraded and perfected. He also published textbooks titled "Processing of Soil" (1940) and "Fertilizers and Fertilization" (1947), moreover, he published over 800 scientific and popular articles and 8 textbooks and teaching aids. Academician Stransky was the first researcher in the country, who correctly defined the "Black Sofia Soil" as "Vertisols" (Stransky, 1947). He published over 140 articles about nutrition and fertilization of plants. Together with Vl. Valkanov Stransky developed bulky research work to do with fertilizer trials in Bulgaria. Many important developments related to general agriculture and processing of soil, crop rotations, erosion, weeds, bacterial artificial fertilizers and modern agriculture, agrometeorology, the role of legumes, essential-oil crops economy country and soil conservation, agricultural machinery, irrigation agriculture, the impact of drought and water properties, human and industrial soils, drought and disease control, cooperative land use and agricultural policy and more are either his personal or his department’s contributions. Academician Stransky remained loyal to his scientific ideas and in the 50’s of the previous century he scientifically and openly rejected the modern trends in soil and agronomic science, which as easily clang to it as later disappeared. True science was everything in his life, he was called by the needs of people to help them. It must be noted that Pushkarov and Stransky had a major role in the creation of the first scientists and specialists of soil science and agriculture. This period is associated with the famous Bulgarian educator and soil scientist Bukoreshtliev Boyko, who first investigated saline soils in Bulgaria and wrote a monograph on them (and Dilkov Raykov, 2007).

II. Second Stage of Development of Soil Science (1947-1969) - the Stage of Rapid Development of Soil Science Associated with the Reorganization of Agriculture in the Country (Garbuchev, 1972)

The second stage can be divided into two sub-stages: the first (1947 -1960) was characterized by founding of two Soil Institutes (one at Bulgarian Academy of Science and one at the Ministry of Agriculture), development of soil science mainly in the horizontal direction, training of soil science personnel, completion of the Average-scale Map in scale 1: 200000 and the monograph "The Soils in Bulgaria”. The second sub-stage (1960-1969) was characterized by merging of the two soil institutes, intensive construction of modern facilities and equipment, approving methodology in research on Bulgarian soil, expanding international relations, completion of the Average-scale Map in Scale 1:400000 under the leadership of Corresponding Member Koinov C. (1968) and the first National Congress of Soil Science in 1969.

The first sub-stage (1947-1960) began with the founding of the Institute of Soil Research at the Academy of Sciences on 26.04.1947, the initiator and director of it being Academician Ivan Stransky (1947-1959) and, in essence, with the decision of the Ministry of Agriculture to perform the famous Bulgarian-Soviet expedition of that year, led by the Soviet academicians I.P. Gerasimov and I.N. Antipov-Karataev , in which all Bulgarian soil scientists of that time took part. The beginning was difficult, but scientists were determined to do their best for the sake of soil science. The Institute was accommodated in the rear rooms of the Natural History Museum of Bulgarian Academy of Sciences and occupied not more than 5-6 rooms, mainly offices. Scientists did not have laboratory facilities, consumables and chemicals, but thanks to Prof. E. Levenson, who had personal acquaintances in East Germany and spoke excellent German, the chemical and microbiological laboratory were later properly equipped. In the forthcoming years a soil physics laboratory, drawing halls for soil mapping, a soil museum and a vegetation house were founded and well-equipped. As time passed by seven departments were founded: Genesis and Mapping, Soil Chemistry, Soil Physics, Soil Biology, Soil Erosion, Soil Fertility, Soil Museum. Once the Institute employed 90 people, it was moved to the new building on the Fourth kilometer. In 1949, the Energohidroproekt soil and irrigation lab with all its staff was associated to the Institute of Soil Surveys and Soil Science of the Ministry of Agriculture (Raikov, Dilkov, 2007). With the expansion of the irrigation system in the country during the period 1948-1958 contracts with the Ministry of Agriculture were signed and extensive soil, irrigation and drainage studies in the areas between the Maritsa River, Old River, and Chirpan Average forest area of 2.95 million acres were carried out by Assoc . Dr. V. Koinov; between Belovo and Svilengrad and the rivers of Vacha, Maritza and Chaya to the foothills of the Rhodope Mountains - an area of 3.17 million ha, the studies were performed by two young scientists - L. Raikov and A. Behar. Out of these studies, Dr. Koinov drew a soil map of the Thracian valley in scale of 1:50 000 million for 3 acres. In 1958, the Department of Genesis and Cartography assumed tasks for large-scale mapping of soils of cooperative farms amounting to 5 million acres between the Sazliyka river in Stara Zagora and the Black Sea (Stransky, 1959). The Soil Map became base material and information for all scientific and business organizations. Thanks to joint efforts of soil scientists and forestry engineers, a national program of afforestation of 10 million ha of forest lands was drafted in 1951.

At the same time the first scientific papers and dissertations on current issues of soil science and agriculture were developed: Prof. Ts. Staykov - Chemistry of the Processes and Dynamics of Nitrate, Prof. E. Yaranov - Characteristics of Llosa, Associate Vodenicharov - Saline Soils and Desalination, Associate El. Kolarova and E. Bratanova - Primary Soil-Forming Process and Mineralization of Groundwater; Associate L. Raikov - Alluvial-meadow Soils, Assistant Prof. N. Ninov - Podzolized Cinnamon Forest Soils, Junior Research A. Behar and Zdr. Arabadjieva - Hydro-physical Properties and Structure of Soil Crust, G. Voinova - Azotobakter, Assistant Professor Biolchev, Assistant Prof. Al. Djingov and PhD N. Onchev - Erosion and Erosion Resistance, M. Milchev and T. Andonov - Fight against Erosion, and Prof. E. Levenson, Assistant A. Kitipov - Fertilizing and Feeding of Plants (Stransky, 1959).

During this phase independent departments of Soil Science at the Higher Agricultural Institute "G. Dimitrov” in Sofia and “V. Kolarov” in Plovdiv were founded, the Department of Meliorative Soil Science to the Civil Engineering Institute and the Department of Forest Soil at Forrestry Technical Institute were founded as well. At the Forest Institute the Department of Forest Soil was opened. In these departments hundreds of specialists were created under the guidance of Academician Ivan Stransky, Corresponding Member V.

Koinov, Prof. M. Penkov, Prof. I. Atanasov, Prof. G. Gurov, Prof. B. Kolcheva, Prof. T. Totev, Prof. V. Donov and many other lecturers. Later, soil science was introduced as a discipline in many universities in the country - St. Kliment Ochridski University of Sofia , the Thracian University, The St. Ivan Rilski South-West University, the Constantine of Preslav University of Shumen, NBU and others. Soil science found a place in secondary schools where the rising generation studied soil types and in vocational technical schools where there already was a discipline called Agrochemistry and Soil Science.

In 1948, the Department of Soil Science at the Central Agricultural Research Institute became a second Scientific Research Institute of Soil Studies at the Ministry of Agriculture called N. Pushkarov. Director was Corresponding Member Ts. Staykov and the staff enumerated 100 people. In the Institute at the Ministry of Agriculture leading specialists in various fields of soil science worked such as Prof. E. Tanov, Prof. Chr. Trashliev , Prof. F. Angelov - Genesis and Geography of Soil, Prof. L. Raycheva - Soil Microbiology, Prof. V. Bakalova - Combating Soil Erosion. As a result of the efforts of the Bulgarian soil scientists, the scientific guidance of Prof. E. Tanov and with the active participation of Prof. C. Koinov, work was started on compiling the average-scale map of the country. During 1951-1960, for a short time the Institute grew to 300 people and was housed in the building in Pavlovo, together with the Institute of Hydraulic Engineering and Land Reclamation. These institutes received the important national tasks related to soil and irrigation and agrochemicals scale soil survey, which tasks both institutes performed successfully. In 1954 a second Bulgarian-Soviet expedition led by Academician I.P. Gerasimov again, was organized to study the genesis, classification and agricultural use of chernozem, zhaltozem, gray and maroon forest soils. Apart from soil scientists the expedition involved collaborators from the Institute of Geography at Bulgarian Academy of Science, headed by its Director - Corresponding Member Prof. G. Galabov.

With the help of the two expeditions several problems of soil science and agriculture were solved:

1. Surveys of the soils in Bulgaria were reassessed, using everything valuable, accumulated over the latest period. In 1964 a modern national soil classification was established, which was converted to the new views on soil-formation processes and the names of different soil types (Koinov, 1964).
2. A new national soil map at scale of 1:200000 was comprised and the monograph "The Soils in Bulgaria" was accomplished, making a contemporary interpretation of the main factors of soil formation. Both were honored with Dimitrov premium (Tanev, 1956; Gerasimov et al., 1960).
3. Modern agro-physical, soil-climatic and irrigation methods in agriculture were widely applied.

The second sub-stage began with the integration of soil science. In 1960, BAS established the N. Poushkarov Central Research Institute of Soil and Farming Practices with Director Corresponding Member Tzvetan Staykov (1960-1963 years). It was established at Bulgarian Academy of Sciences by combining the Institute for Soil Research at the Academy, the N. Poushkarov Research Institute of Soil Studies at the Ministry of Agriculture and the Department of Soil and Crop Rotation at the Institute of Plant in Bulgarian Academy of Sciences headed by Academician Konstantin Pavlov. In 1962 the joint Institute with Director

Corresponding Member Ts. Staykov transferred to the new Academy of Agricultural Sciences in Bulgaria and after a while (1965) it was already the present N. Poushkarov Institute of Soil Science. During this substage, until 1968 new large-scale studies of farmland in the country were made and the national soil map in 1:400000 scale was completed (Koinov et al., 1968). Detailed studies on food and water regime of soils were carried out. The beginning of extensive soil and geographical network of field experiments was set. Meanwhile the following tasks were defined to be solved by Bulgarian Soil Science:

1. Study of soil resources, genetic processes and improvement of the classification of soils in Bulgaria.
2. Adjusting of soil fertility on the basis of high chemicization in the main soil types in the country.
3. Development and implementation of a system of measures into practice to combat erosion.
4. Amelioration of soil with unfavorable properties or properties.

In the second stage two cycles of systemic agrochemical research on soil were conducted for which a certain role played Prof. K. Enikov being an agrochemist and director of the institute during the period 1951-1960. The first research cycle (1956-1960) on availability in the soil of absorbable forms of nutrients covered 38 million acres and revealed the real possibilities of our land fund (Enikov, 1979). The second cycle (1966-1979) conducted with the aid of modern methods, covered about 45 million acres of agricultural land and confirmed the right way for intensification of agricultural production in terms of mineral fertilization and the need to progressively increase the size of this event (Enikov, 1979). Accelerated development in the first decade of this stage was acquired by the research on meso and trace elements, whose role and importance strongly rise in parallel with the increasing scale of fertilization with microelements. On 01/01/1963, at the Academy of Agricultural Science a Central Laboratory for Agrophysical Research was opened which was consistently managed by Prof. R. Andreychin, Prof. Dr. A. Ivanov and Prof. Dr. Ivan Varlev. От 01.01.1976 the lab moved to N. Pushkarov Institute of Soil Science becoming today's Agrophysics department. During this stage, in the early 1966, the magazine Soil Science and Agricultural Chemistry (since 1992, "… and ecology:") was based with senior editors ever since: Prof. K. Enikov, Prof. I. Garbuchev, Prof. P. Ivanov, Prof. D. Slavov, Prof. M. Penkov, Prof. B. Georgiev, Prof. M. Teoharov.

II. Third stage - Modernization of the Equipment, Strengthening of International Relations and Recognition, Advanced Science Servicing of Agriculture (1969-1989)

In 1963, Professor Ivan Garbuchev was appointed as director of the Institute. He was a prominent scientist and organizer of soil and agricultural science. He made major structural improvements. He created a new department of Scientific Service Fund Management for Large-scale Soil and Agrochemical Studies with Senior managers M. Jolevski, Ph.D. ASA. Hadzhiyanakiev and Research Georgi Dimitrov. There were also two new departments – one for Soil and Crop rotation with Head E. Dzhumalieva and one for Field Trials, Hospitals and Statistics with Head Prof. E. Ilkov. The Department of Soil Fertility was renamed Department of Agrochemistry. The remaining 6 departments were headed by Prof. V. Koinov (Genesis, Geography and Soil Classification), Prof. C. Galeva (Soil Physics), Prof. L. Raikov (Soil Chemistry), Prof. T. Palaveev (Agrochemistry), Prof. G. Voinova (Soil Biology) and Prof. A. Biolchev (Soil Erosion). In 1969 the first international contract with FAO for 1,2 million dollars was prepared and signed, led on Bulgarian side by Prof. Ivan Garbuchev and by FAO - Prof. El Gabbana. However, the government was granted BGN 5 million for a new building complex with the growing house fitotron, and School of Postgraduate Studies which was opened in 1972 by the President of the State Council Todor Zhivkov. In the same year the Institute was awarded the Dimitrov Prize. FAO provided funds for new equipment and training of specialists in the world's leading schools. New scientific methods and analyses – radioisotopic and radiometric, X-ray-structural, differentially- thermal and electronic-microscopic – were adopted. The Institute was visited by international government delegations and world-renowned specialists in the field of soil science - Academician Gerasimov, Acad. Turin, Acad. Chernesku, Acad. Filipovski, Prof. Hartke, Prof. Jenny, Prof. Friedland, Prof. Zonn, Prof. Rozanov, Prof. Baumgardner, Prof. Arnold, Prof. Sobolch, Prof. Chirich, Prof. Tavernier, Prof. Dyudal, Prof. Auber, Prof. Elgabali, Prof. Plahi, Prof. Evald, etc. Prof. Ivan Garbuchev had the greatest merit for the Institute to become an international training center for post-graduate students of the countries of Asia, Africa and Latin America; to implement and coordinate a number of important research activities at national level and to participate in the development of international projects and contracts. Moreover, geographical soil and agroenvironmental zoning of the country was made (Ninov et al., 1972, Jolevski et al., 1980). The geographic-soil network of experimental fields and stations in the country (more than 30) was expanded and the laboratory work on agrochemicals service was centralized by closing some of the laboratories in the country. As well as establishing the Data Center and the Central Agrochemical Laboratory, an automated system for making recommendations for fertilization was created (Raikov, 2001). The whole arable area was comprised in a periodic agrochemical control and the large-scale soil mapping at 1:25000 and 1:10000 on agricultural land was completed. An agricultural grouping of soils in Bulgaria was accomplished, which played an effective role in crop-growing (Jolevski et al., 1974). For speeding the development of agricultural production in different areas regional soil maps of several counties were made (Angelov, 1975). The Institute was recognized as a science center with important services to a modern and profitable agricultural production. In the 60,s the different aspects of soil science were accredited, employing well-trained researchers, who left deep traces in its development:

- Genesis, geography and soil classification (Hr. Trashliev, V. Koinov, E. Angelov, N. Ninov, T. Boyadjiev, Sl. Krastanov, E. Fotakieva, T. Andonov, J. Staykov, I. Kabakchiev, P . Treykyashki);
- Soil Physics (V. Galeva, A. Ralchev, I. Vatralov, T. Ovcharova);
- Soil Chemistry (L. Raikov, G. Hinov, P. Vodenicharov, A. Behar, K. Paskaleva, St. Ganev, Z.. Varbanova, G. Hubenov, D. Bratanova, S. Mirchev, Y. Kavardzhiev);
- Agrochemistry (T. Palaveev, D. Dinchev, M. Milcheva, E. Neykova, A. Kitipov, N. Mitreva, Y. Bachvarova, D. Stoyanov, N. Patarinski, E. Ikonomova, S. Nesterova, M. Mincheva);
- Soil Biology (G. Voynova, L. Raycheva, B. Todorova, P. Petkov, D. Bakalivanov);
- Soil erosion (A. Biolchev, V. Bakalova, Al. Djingov, N. Onchev, S. Nikolov);
- Soil and crop rotation (D. Dzhumalieva, F. Todorov, K. Stoynev, P. Pavlov);
- Field experiments, stations and statistics (D. Ilkov, V. Barov, S. Lichev, Y. Shanin, T. Gruev, S. Atanasov, P. Petrov, M. Benevski, T. Konov).

In 1973 a new basic scientific direction - Programming of Yields was introduced in the Institute. It was originally created in 1971 in the Faculty of Agronomy of the Academy of Agricultural Science - the Department of Plant Physiology and it gave a complex nature of soil research. The management of agrochemical service of agriculture, which concentrated all the chemical laboratory work and computing equipment, allowed making recommendations by tabulograms for blocks and crops across the country. During the period 1973-1974, being Director of the Institute and Head of the Permanent Working Group on Fertilization to the Commission of Agriculture in the Union for Economic Cooperation, Prof. Cyril Enikov significantly accelerated the work on agrochemicals characterization of the major soil types in Bulgaria, in conjunction with the need for fertilization of soil. In the 70's a Long-term Program to Combat Erosion and a National Program for Improvement of Fertility of 15 million ha of low productive lands were developed and implemented with the participation of the Institute for Forest and The Forestry Engineering Institute. In 1976 the N. Pushkarov Institute was incorporated into the structure of the National Agro-Industrial Union. Decree № 8 of 09.02.1976 extended the objects of activity and until the year 1978 it bore the name Institute of Soil Science and Agrochemistry. After that it was renamed to Institute of Soil and Programming Yields. From 1982 onward the Institute was again in the system of the Academy of Agriculture. With the appointment as Director of Prof. L. Glogov in 1977, the objects of activity of the Institute were greatly expanded to 5 directions and 17 departments:

I. Direction - Balance, Preservation and Reproduction of the Soil with four departments - Genesis and Classification of Soils, Soil Chemistry, Soil Microbiology, Soil Erosion.

II. Direction - Increasing and Regulation of Fertility with four departments - Agrochemistry, Mineral Nutrition, Soil Cultivation and Crop Rotation, Statistics and Mathematical Modeling.

III. Direction - Programming of Yields with four departments - Crops, Productive Processes and Mathematical Modeling, Parametersetting of Soil Conditions and Photosynthetic Activity, New Technological lines in programming yields.

IV. Direction - Agrophysical Research with three departments - Soil Physics, Biophysical and Microclimatic Studies, Instrumentation Design Bureau.

V. Direction - Soil and Agrochemical Service to Agriculture with two departments - Soil Mapping and Soil Analysis Control.

State and government authorities placed the Institute to much higher requirements, utilizing the scientific capacity and the broad interdisciplinary themes. A team of scientists developed a scientific program, models and agroecological engineering projects for programmed yields of main agricultural crops - wheat, corn, barley, potatoes and others. The program was consulted and assisted by Academician Shatilov. To solve the forage and food problems some alternative crops and varieties were introduced whose technological characteristics were checked in the experimental facilities of the Institute. For general support of applied research at this stage some high-level scientists from the Institute of Wheat and Sunflower in General Toshevo joined N. Pushkarov Institute. There were working groups that directly supported the farms on-site. International agreements on issues of programming yields were signed with the USSR, USA, Holland and other countries. In the mid 80's an international treaty with the United Nations Environment Program (UNEP) was signed to develop a project for conservation and development of mountain ecosystems, managed by Prof. Sl. Krastanov and Prof. A. Behar. In 1981, 1982 and 1983, FAO conducted three consecutive meetings of international and Bulgarian soil science community at the Institute and developed the basic principles and rules for drawing the world reference base for soil resources. The numerous and detailed studies allowed the soil classification scheme in Bulgaria to be improved (Jolevski et al. 1983). An agro-ecological regional map was composed and the country was allocated to 40 agricultural and 10 forest areas (Jolevski et al., 1980). During this stage there was active cooperation with scientists from the Institute of Economics and Organization of Agriculture in conjunction with the assessment and cadastre of land. The joint team from both institutes developed a new methodology for categorization of agricultural land (Petrov et al., 1988).

IV. Stage - Stage of Reforms, Changes and Integration into the European Research Area (from 1989 until today).

This stage is characterized by withdrawal of important scientific and practical parts of the Institute and limiting its activities, also reduction of the personnel several times. Nevertheless, there has been successful integration with the European Research Area. For two decades the number of personnel of the Institute has dropped from 1041 to 170, and the academic team has been reduced from 214 to 82 scientists. Some subjects have dropped out due to lack of equipment or young scientists. Scientists' pay is low and young people do not consider that science is their vocation. Over the past 15 years the personnel of the Institute has been working under difficult conditions in winter months. Reagents, chemicals and consumables are purchased with funds generated by our own revenues or finance from European projects. Over the past two years funds from European projects have been used to cover part of the maintenance of the Institute. Experimental studies in some of the research facilities have reached critical levels or are no longer performed. However, many topics are reevaluated on a qualitatively new level, according to European standards. Despite the difficulties of the time, the 100-year tradition of soil science and the vital necessity of its scientific products for practice and European structural units have now established new cardinal tasks resulting from the processes of harmonization and globalization of science and problems in the country. Therefore, at this stage the management of the Institute and other entities outside has been trying to preserve the traditional academic subjects, which continue to be sought at the national and international level.

In 1986 Prof. Dr. Valio Valev was appointed as Director of the Institute and he, with a little break (1992-1995), was in charge of its personnel until 2000 (Vichev, 2007). This period was characterized by a gradual reassessment of priorities, objectives and tasks of soil science, according to the changes after 1986 and especially after 1989. A new methodology for categorization and cadastre of agricultural land which was developed in the institute was used for the compilation of cadastral and farm maps. Scientific and applied activities of the Institute were associated with large-scale mapping of soils in 1:10000 and 1:50000 scale. A new classification and diagnostics of soil in Bulgaria in connection with the land partition was created including scientists from the Institute of Forest and Forestry University (Penkov et al., 1992). After 10 years of work an Atlas of Soils in Bulgaria was composed (Koinov et al., 1998). There has been active work on an assessment of environmental potential of soil and crop suitability. The scientific agro-environmental field has been validated and this led to the necessity the Institute to be renamed by a decision of the Presidium of the Academy of Agriculture from 19/07/1990 as Institute of Soil Science and Agro-environment, without its traditional academic subjects being compromised. A FAO project was won and it was managed by Prof. Dr V. Valev and Prof. Dr. T. Boyadjiev. Prof Valev began training soil scientists from the Mediterranean and the Middle East as the lectures were read in French for students from Algeria, Lebanon, Morocco and Tunisia and in English for students from Egypt, Jordan, Libya, Iraq, Cyprus and Syria.

From 1992 to 1995 Director of the Institute was Prof. Dr. Dimitar Stoychev. Under his leadership a PHARE project for 1,2 million ECU was won, which led to the creation of a modern physico-chemical and agrochemical laboratory for soil, vegetation and water, which was later transferred to the National Service of Agricultural Protection and then it was three more times subsequently wrongly based. Attribution of a "political profile" of soil and agricultural land by nonprofessionals and pseudo-environmentalists at the beginning of the period of democracy strengthened the environmental direction at the Institute. National and international level contracts and projects of pollution of soil and water with heavy metals, radio-nuclides, petroleum products and waste products were signed. A joint US-Bulgarian project for establishing the level of pollution of the Yantra river, run by Dr. E. Stoychev supplied funds for the purchase of new equipment. The Institute led a successful fight to deny the current misconception of soil pollution by fertilizers and pollution of rice fields in Thrace with heavy metals. American scientists were invited, who shared the views of the Bulgarian soil scientists of the absence of local pollution in the rice fields and total soil pollution in the country. Scientists from Bulgarian Academy of Sciences and the Institute developed a project to create a national park "Central Balkan", funded by the National Park Service of the U.S.A. Bulgarian soil scientists participated in comprising the Soil Map of Europe.

At the beginning of 2001 Prof. Dr. Toshko Raichev was appointed as Director of the Institute and from March the same year until the end of 2003 Director was Prof. Dr. Martin Banov. From 2004 to 2008, the Institute was headed by Prof. Dr. Nicola Vichev. This eight-year period is characterized by constant reforms imposed by the changes. We must appreciate the work of the three directors who were able to preserve the validated for years on basic scientific themes of the Institute. During this phase international cooperation with many countries from Europe and the Balkans has continued to expand. The Institute participated in an international project on environmental assessment of soils. The accredited Test Laboratory in Radioecology and Radioisotope Studies, which by order of the Ministry of Agriculture was declared National Reference Laboratory in 2007, has been sufficiently improved. A comprehensive assessment of environmental agro-production potential of the agro-tech-parks in the country has been created and the structure of agricultural production has been optimized. (Bachvarova et al., 2005). Bulgarian soil scientists participated in the development of the Soil Atlas of Europe.

From 2008 to 2011 the Institute was headed by Prof. M. Teoharov. For the successful implementation of its activities three scientific programs were defined - Balance, Reproduction and Conservation of Soil Resources; Maintenance and Increase of Soil Fertility and Agricultural Research Services; Integration and Innovative Directions in Research on Soil and Land. Related approaches and methods have also been defined for the sustainable development of soil science in the Institute. The three programs include implementation of 11 scientific research projects of the regular curriculum, 4 international projects and an international contract (with a total value of 400 thousand euro), covering the main priorities of the Institute and the European Research Area. Based on surveys of multi-yearly research of many soil scientists Reference Database of Soils in Bulgaria has been developed and published, which set a new benchmark in the use of soil information for the national and international tasks. This database is used for assessment and strategic development of geo-information services to European soil data at the moment (Teoharov, 2009; Kercheva 2009-2012). Strategically important works have been out of print recently - "The Soils and Land in Bulgaria - National Secular Wealth" (Teoharov, 2011), "Risk of Soil Erosion in Bulgaria and Recommendations for Soil-protection Agricultural Land Use" (Rousseva et al., 2010). Vulnerable nitrate areas in the country have been defined; rules of good agricultural practices have been developed (Stoycheva et al., 2008). A complex system of scientific service to farmers across the country was built and it was developed in the Information, Consultancy and Innovation Center with Central Research Laboratory, Bureau of Recommendations, Advice, Opinions, Expertise and Design, a mobile group for field surveys and sampling, an expert group to read the analyses and give recommendations and opinions, input and output register for sampling and analyses and mapping laboratory. Only in 2010 the Central Research Laboratory received 2506 soil samples and 1566 water samples and 23,443 definitions were made. 206 farmers were serviced and 159 technological solutions, recommendations and opinions were given. In practice, this is a national service system for agriculture and environmental protection.

Applied science and implementation activities are based on the work in established scientific fundamental directions - Soil and Genetics, Environmental Soil, Agrophysics, Agro-environment, Agrobiology, Agrotechnics and Radioecology. Essential scientific trends are at the basis of the technologies and innovative solutions, namely:

- Reference data base for soils in Bulgaria;
- Complete diagnostic and agro-production assessment of soil and climatic and agroecological potential of soil resources for the effective cultivation of crops;
- Models for establishing, maintaining and improving soil fertility;
- Erosion control and soil-protection technology in agricultural land use;
- Technological solutions for the remediation of contaminated land and amelioration of acidic, saline, disturbed and eroded soils;
- Methods and regulations for utilization of waste products from households, industry and agriculture;
- Methodological developments for the production of biogas from waste products;
- Biotechnological methods and solutions to increase yield and quality of plant production;
- Advanced systems and technologies for organic and sustainable agriculture;
- Checking and development of energy technologies and alternative crops;
- Radioecological and radiometric monitoring of soil, water and agricultural produce;
- Norms, regulations and directives for the harmonization of Bulgarian legislation with the EU in the field of agriculture and ecology;
- Training of farmers and specialists in the field of soil science, agriculture and agro-environment.

From 2011 to present moment Directors are Prof. Ds N.Dinev. Prof. Dr T. Mitova and Prof. Dr S. Rousseva. This is a period of reforms and confirmation of new Science directions. In 2012 the Institute is united with the Institute of Mechanization and Hidromelioration and the Institute of Plant Protection. It was renamed as Institute of Soil Science, Technology and Plant Protection.

At the final stage of its development the Institute has held its equivalent position in the ERA. Research projects within 5-th , 6-th and 7-th Framework Programs of the European Commission, the scientific and technological development Inko- Copernicus programs, COST programs, E-content + , Program for Southeast Europe have been completed. Bilateral cooperation with Belgium, Germany, Greece, China, Russia, USA, France, Netherlands, Poland, Hungary, Slovenia, Slovakia, Cyprus and more has been extended. We are respected partners in the European Soil organizations and networks such as the European Soil Bureau, European Association of Soil Scientists, the Centre for Drought Management in Southeastern Europe, International Centre for Theoretical Physics and others. Some of our young scientists currently specialize in these reputable centers. World renowned scientists have been visiting the Institute and working with our team – for example Prof. Targulyan, Prof. Montanarela, Prof. Blum, Prof. Bech, Prof. Mermut, Prof. Uenema, Prof. Teridze, Prof. Urushadze, Prof. Bielek, Prof. Kapizhotis, Prof. Mikkeli, Prof. Shein, Prof. Paschalidis, Prof. Ttsadilas, Prof. Varalay, Prof. Vaca, Prof. Zdruli, Prof. Haynos, Prof. Jozefachiuk, Prof. Sokolovska, Prof. Nemetch, Prof. Kozak, Prof. Dimitru, Prof. Gabrielle, Prof. Horn, Prof. Haigh, Prof. Lal, Prof. Mesič and many others. Important issues of bilateral cooperation have been discussed with the Director of the Dokuchaevski Institute Prof. N. Khitrov. Scientists from the Institute participated in the teams developing the European Strategy for Soil Protection, the national position of Bulgaria on the Adoption of European Directive for Soil Research Projects in support of the European strategy on soil protection, the CAP of the EC to reduce greenhouse gas emissions of agricultural lands, the INSPIRE directive management, standardization and coordination of spatial database, protection of waters against pollution caused by nitrates from agricultural sources and biogas from waste products from livestock.

Our scientists are in aid of legislation and government. Working groups from the Institute participated in the development of Land Act, Law on Soil Protection, the National Action Program for Sustainable Land Management and Combating Desertification in Bulgaria (2007-2013), National Agro-ecological Program of Bulgaria (2007-2013) within the Program for Rural Development, Environment Operational Program (2007-2013), in the drafting of regulations, standards and others at Parliament, ministries (Ministry of Agriculture and Food, Ministry of Environment and Water, Ministry of Regional Development), intergovernmental and parliamentary committees.

For its contribution to the development of Bulgarian science and active international activity, the Institute was awarded a Golden Book and Golden Seal in 2009 by the Council of the European scientific and cultural community and in 2011 bigest state reward – Honorary Sign of President the Republic Bulgaria.

Today the Bulgarian Soil Science has one hundred and fife years. The Institute, named after its founder Nichola Pushkarov, a pioneer and generator of new ideas, and many smaller structural units in institutes and universities of the country are key holders and custodians of soil science and distribute knowledge acquired in the institute. The Bulgarian Soil Science company, led alternately by Academician Ivan Stransky, Prof. Ts. Staykov, Prof. I. Garbuchev, Prof. L. Raykov, Prof. M. Jolevski, Prof. T. Boyadjiev, Prof I. Kolchakov, Prof. R. Dilkova and Prof. M.Teoharov has had an important role to its international prestige and social significance.

It has been a century in which Bulgarian soil science has emerged as an important branch of biological science and has become a science of undoubtedly great public importance. In its 100-years' history, soil science has always been in service of people closely related to problems of soil fertility, of land and its efficient use and conservation. Soil science is a capital that must not be lost. In all stages of its development in its foundation soil science has always been a recent addition to basic scientific direction, which shows its inseparable connection with practice. When new needs of society emerge, then new research directions are created. Soil science as a leader and at the heart of agrarian science is destined to go 10-15 years ahead, to give strategic outlines, according to the needs of people, the agro-economic and social development of the country.

With its interdisciplinary nature of research and many wide and varied research subjects, soil science disposes of current research and innovations, which have always been sought by a wide range of users. Therefore, management of soil and land resources must become a consistent and sustainable government policy, and N. Poushkarov Institute of Soil Science must be maintained as a national scientific and methodical base and focal point for scientific services to agriculture, ecology and environmental protection. Soil science, despite the difficulties of the time, has scientists of high human potential, scientific capacity, necessary knowledge and competence of quality and up-to-date solving of tasks, performed at national and international level.

The history of the Institute of Soil Science is in fact history of Bulgarian soil science itself, for Pushkarov Institute is bearer, guardian and propagator of this science and it is in turn an inspirator of its scientists.

May the science of soil and land live through the future centuries! Let us worship the first who put its foundations and all the others who upgraded it with more consistency, new ideas and courageous work! Let us pay tribute to all those who in various stages of its development remained true to themselves, boldly defending their scientific ideas! Let us thank the galaxy of scientists who gave their modest personal contribution to the preservation of our national and secular wealth - soil and land!

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Cambisols Classification in „Central Balkan” National Park

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Abstract

Analysis of the Cambisols classification on the territory of the Central Balkan National Park has been carried out. The assessment of the available information has revealed the insufficient level of knowledge and use of outdated criteria for soil definition. The presence of *Dystric Cambisols, Eutric* and *Dystric Regosols, Haplic Umbrisols*, *Rendzic Leptosols*, *Haplic Fluvisols* has been determined in separate studies. A detailed study is required in order to define the soils and update their classification. This will contribute to the proper planning of the necessary measures for soil protection for the different types of land use.

Keywords: *Umbrisols, Cambisols, Regosols,* base saturatuion, national park

State-of-the-art

The Central Balkan National Park is one of the most valuable and the largest protected areas in Europe – second category according to International Union for Conservation of Nature. It has an area of 72021.07 ha and it is the second largest national park in Bulgaria. The Park contains rare and endangered wildlife species and communities, self-regulating ecosystems of biological diversity, as well as historical sites of global cultural and scientific significance.

The soils on the territory of the Central Balkan National Park are insufficiently studied. The analysis of the soil map (fig. 1) of the park Management plan (MP, 2014 – 2023 project, pp. 48; pp.199) shows that *Cambisols* are present in the forest areas (96.9% of the forest territories), while *Umbrisols* are distributed almost entirely in the areas of grasslands and meadows (about 55% of the park area). The *Luvisols* are present on 2.1% of the park territory, the *Cambic Umbrisols* – on 1% of the territory. The portion of *Rendzic Lepthosols* is insignificant (MP, 2001-2010 pp. 21-24; MP, 2014-2023 project, pp. 29-30).

The analysis of the soil subtypes shows that 32.1% of the *Cambisols* are “dark”, 56.6% - “transitional”, and 11.3% are “light” (MP, 2001-2010). The maps, necessary to determine their area and distribution, can be found neither in the park management plans (MP, 2001-2010; MP, 2014-2023), nor in the scientific literature.

An attempt for correlation between the national soil classification of 1980 and the basic classification of 1992 has been made in the project for updated management plan (MP, 2014-2023). It is stated that the *Eutric Cambisols* occupy 32.1% of the total *Cambisols* area, which is practically equal to the area, occupied by the subtype “dark” *Cambisols* of the MP (2001-2010). Since it is not clearly specified, the conclusion that the remaining area, occupied by *Cambisols*, refers to the *Dystric* can be made by default. In practice, the areas

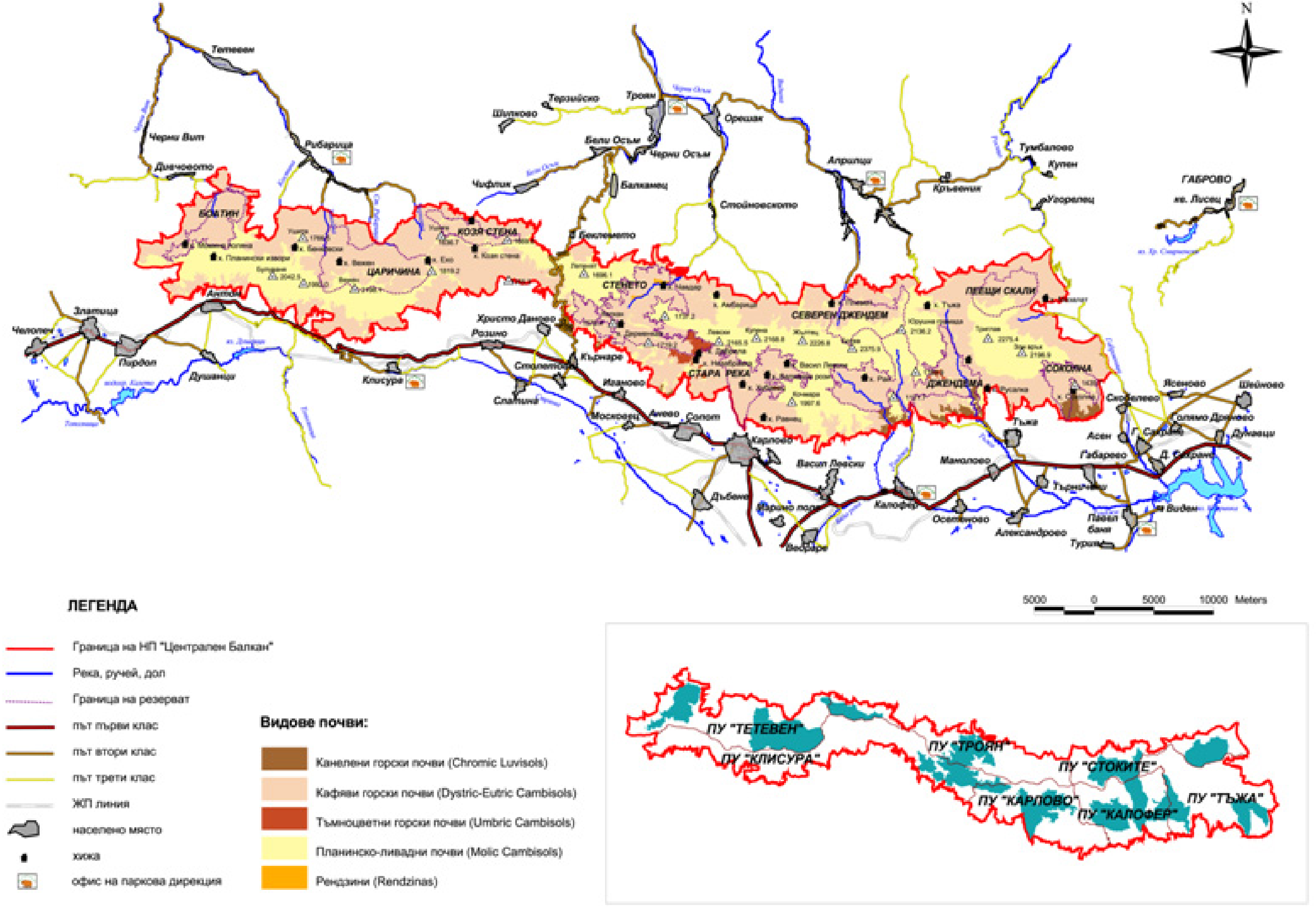

Figure 1. *Soil map of „Central Balkan" National park (MP, 2014 – 2023 project)*

Table 1. *Investigated soil - location, land use, altitude, parent materials, soil group and qualifier*

Site №	Latitude and Longtitude	Land use	Altitude (m)	Parent materials	Soil group (WRBSR, 2006)	Qualifier (WRBSR,
1	42°46' 34.9" N 24°21' 42.4" E	forest	1503	gneiss	Cambisols	Dystric
2	42°46' 26.8" N 24°39' 07.3" E	grassland	1445	schist and sadstone	Cambisols	Dystric
3	42°46' 44.9" N 24°37' 50.3" E	forest	1440	schist and sadstone	Cambisols	Dystric
4	42°41' 44.0" N 24°59' 42.0" E	forest	1539	granite	Cambisols	Dystric
5	42°41' 02.4" N 25°06' 57.3" E	forest	1247	schist	Cambisols	Dystric
6	42°46' 52.2" N 24°20' 01.3" E	grassland	1536	granodiorite	Umbrisols	Haplic
7	42°46' 53.3" N 24°36' 14.6" E	grassland	1529	schist	Umbrisols	Haplic
8	42°46' 04.0" N 24°39' 51.0" E	grassland	1635	schist	Umbrisols	Haplic
9	42°42' 36.6" N 24°58' 52.9" E	grassland	1877	weathering materials from granite	Umbrisols	Haplic
10	42°41' 20.0" N 25°06' 50.0" E	grassland	1384	weathering materials from schist	Regosols	Dystric
11	42°44' 04.7" N 24°29'10.9" E	grassland	1165	weathering materials from granite	Regosols	Eutric
12	42°44' 42.0" N 24°36'46.0" E	grassland	1270	weathering materials from gneiss	Regosols	Eutric
13	42°44' 46.0" N 24°36' 54.0" E	forest	1272	weathering materials from gneiss	Regosols	Eutric
14	42°40' 51.0" N 24°57' 35.0" E	grassland	1100	limestone	Leptosols	Rendzic

of the “transitional” *Cambisols* (56.6% of the total area) are not included in this description. It should be noted that the correlation with the classification of 1980, as well as with other similar classifications, is specified in the basic soil classification (Penkov et al., 1992), but the term “transitional” is not mentioned for the *Cambisols* , i.e. there is no option for a correlation with other classifications. This classification defines “common” *Cambisols* .
The term “transitional” *Cambisols* is absent from the Soil Forest Science textbooks by Georgiev (1959) and Donov (1979; 1993), as well as from the Soil Science textbooks by Gyurov et al. (1969), Koynov (1980) and Penkov (1996). In “Forest Soil Science” Donov (1979) made an overview of the *Cambisols* classification, returning back to 1948, when the Russian-Bulgarian expedition was carried out under the leadership of Gerasimov and Antipov-Karataev, but he did not mention the presence of the transitional *Cambisols* subtype. The term “transitional” is used in the Forest management plans, developed for the entire forest area of the country. The determination of the subtypes is carried out by the depth of the surface horizon as follows: “dark” - 25-40 cm, “transitional” - 10-20 (25) cm and “light” – less than 10 cm. This longstanding practice separated the *Cambisols* from the basic soil classification (Penkov et al., 1992) and from the global classifications.

In 2011 (Raikov, et al.) an update of the Instruction for identification and mapping of the forest habitat types, applied in the Forest management plans, was made. The correlation between the national soil classification of 1980 and the basic classification of 1992 was adopted. In this classification the “common” *Cambisols* are defined as *Eutric* and the “light” ones as *Dystric*. No equivalents were determined for the “dark” subtypes. Although this actualisation was a step forward, the thus obtained correlation was too inaccurate. For example, the subtype “light” *Cambisols* are low in humus, eroded and in such cases the influence of the soil-forming materials, which are actually the source of the soil base richness, is enhanced on the surface. The probability that such soils are *Dystric* is small, even for those, developed on granite. Actually, defining at a soil qualifier level can be made only on the basis of the information for their base saturation, obtained from laboratory analyses. The compliance between “dark”, “light”, *Eutric* and *Dystric Cambisols* is not possible due to the fact that colour can be used as a diagnostic feature only for humus content but not for base saturation.

The pilot studies carried out in 2015 on the territory of the park and supported also by the data of laboratory analyses show the presence of other soil units (Malinova, 2015, Malinova, 2016) except those, specified in the Management plans. The summarized information of their analysis is given in Table 1. The basic principles of WRBSR (2006) have been applied (“The classification of soils is based on soil properties defined in terms of diagnostic horizons, properties and materials The selection of diagnostic characteristics takes into account their relationship with soil forming processes”). The classification of WRBSR (2006) has been applied and the qualifiers have been determined, which allow to define two tiers of categorical detail. In forest areas, as well as in the pasture and meadow, regardless of the color of the surface horizon, the presence of *Dystric Cambisols, Eutric* and *Dystric Regosols, Haplic Umbrisols, Rendzic Leptosols, Haplic Fluvisols* has been determined. The results show that a detailed study in park territory is required in order to define the soils and update their classification.

The analysis of the available information about the soils on the territory of the Central Balkan National Park has shown the insufficient level of knowledge and use of outdated criteria for soil diagnostics. A detailed study is required in order to define the soils and update their classification. The proper soil definition will contribute to the better planning of the necessary measures and procedures for soil protection for the different types of land use.

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Organic Matter Content and Composition of Soils with Stagnic Properties from Bulgaria

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Abstract

This paper deals with the contents and composition of organic matter in soils with stagnic properties. Study on the content and composition of soil organic matter, shows that these soils have low content of organic carbon. Soil formation proceeds under influence of acid plant residues from forest and seasonal waterlogging caused by the surface stagnating waters. The total carbon content decreases sharply in subsurface horizons. The amount of fulvic acids (C_f) prevail over humic acids (C_h) and type of humus is humic-fulvic and fulvic.

Key words: Soil organic matter (SOM), humus, fulvic acids, humic acids, Stagnosols, Planosols

Introduction

Soils with stagnic properties are spread mainly in Central Bulgaria close to the *Stara Planina* (Balkan mountain) and Sredna Gora. According to the Bulgarian soil classification these soils are considered as Pseudopodzolic soils (Koinov et al., 1964; Penkov et al., 1992). They occupy about 9.75 % of the territory of Bulgaria (Shishkov et al. 2014). According to Ninov and Teoharov (1990), the area of Pseudopodzolic soils with surface waterlogged in Bulgaria is 180 000 ha. The World reference base for soil recourses - WRB (IUSS Working Group WRB, 2014) classify these soils as Planosols and Stagnosols. These two soil types are spread over secondary-sediments, highly eroded granite, andesite, sandstones and metamorphic rocks, mainly acidic (Koinov et al., 1998). They occur on flat to gently sloping lands with a cool and temperate humid climate. Soil formation proceeds under the influence of acid plant residues from forests (mainly oaks) and seasonal waterlogging caused by the surface stagnating waters. Soil formation consisted of some pseudo-clay formation processes as, waterlogging, leaching, and clay formation.

Studied Stangosols and Planosols have stagnic properties in depth. They are low productive due to the unfavorable soil properties. These soils have one of the lowest content of organic matter in the country. Average humus content is about 1 - 1.5 % cultivated soils, up to 2% under forest. The organic matter stocks in the one-meter layer do not exceeds 2 t per hectare in uncultivated lands and 1-1.5 t/ha in agricultural areas (Koinov et al. 1998). Conditions of humus formation and humus accumulation of Stagnosols and Planosols are unfavorable (Artinova, 1977; Gushevilov and Filcheva, 1988; Ilieva, 1986; Ilieva and Grishina, 1986; Kolchakov, 1978; Moshev, 1989; Filcheva, 1976; Filcheva et al., 1994; Filcheva, 2007; 2015, Petkova, 2014). The soil organic matter determination as a diagnostic feature of the soil and data on organic matter composition of this soil group were published earlier (Boyadgiev et al, 1994b, 1994c, Filcheva and Boyadgiev, 1997). The organic matter composition is characterized by the low content of humic acids (C_h) compared with some soils in Bulgaria. However, fulvic acids (C_f) are presented in significant quantities, which determine the very low C_h/C_f ratio. The humus type is fulvic along the soil profile depth ($C_h:C_f <0.5$). The content of humic acids is low and sharply decreased along the profile's depth (Artinova, 1977). In the middle part of the soil profile humic acids are missing and at the bottom the amount is increasing. Morphologically established dark color in this part of the profile is explained with increasing of humus content, probably as a result of movement of mobile humic acids in this horizon and bind with calcium in less mobile compounds (Filcheva, 2015). Due to the low pH and seasonal surface waterlogging, the degree of humification in these soils, especially in the area of waterlogging is low, with the exception of the upper part of eluvial horizon. Humic acids in the surface horizons are represented as ”free“ and bind with the mobile forms of R_2O_3. The content of aggressive fulvic acids fraction in the SOM composition is high (12-20 %), mainly in the areas with intensive periodic of gleyic processes (Filcheva, 2015). These soils are very poor in organic matter and nutrients the biological activity is also low.

The aim of this paper is to examine the present state of soil organic matter (SOM) of soils with stagnic properties and their humus quality, quantity and composition.

Materials and Methods

Three soil profiles were studied in central part of Bulgaria.

Profile 1, is located at N 42˚39.373'; E 027˚48.046', (240 m elevation) near to village of Banya (Varna district). Soil moisture and temperature regimes are mesic and udic. The land use is agricultural with crop rotation. According to WRB 2014 soil type is classified as Luvic Gleyic Stagnosol (Aric, Chromic, Clayic).

Profile 2, is located at N 42˚36.31.6'; E 023˚37.069', (638 m elevation) near to village of Novi Han (Sofia district). Soil moisture and temperature regimes are mesic and udic. The vegetation is forest (Oaks). According to WRB 2014 soil type is classified as Dystric Luvic Umbric Planosol (Chromic, Epiclayic). Data are taken from Boyadjiev et. al. (1994a).

Profile 3, is located at N 42˚24.751'; E 025˚01.220', (312 m elevation) near to village of Zlatosel (Plovdiv district), Soil moisture and temperature regimes are mesic and xeric. The vegetation is forest (Oaks). According to WRB 2014 soil type is Epidystric Luvic Albic Planosol (Chromic, Epiclayic, Bathycalcaric). Data are taken form Hadjiyanakiev et. al. (1981).

Content and composition of SOM in the studied profiles are determined with the modified Tjurin's method [oxidation with a solution $K_2Cr_2O_7/H_2SO_4$ in a thermostat at 125^oC for 45 min, in the presence of a catalyst Ag_2SO_4 and titration with $(NH_4)_2SO_4$. $FeSO_4.6H_2O$ and the Kononova – Belchikova's method (Kononova, 1966; Filcheva and Tsadilas, 2002)].

Results and Discussion

The study on the content and composition of SOM confirm previous results that a soil with stagnic properties has low content of organic carbon (Filcheva, 2015). Data show the following: content ranges between 0.7 and 1.7 % in the surface horizons although these profiles (2 and 3) are under forest (Table 1).

Total carbon content decreases sharply in subsurface horizons. This is typical for soil such as Planosols (Figure 2 and 3). In profile 1, organic carbon is also low but decrease in depth is more gradual (Fig. 1).

Table 1. *Content and composition of soil organic matter. Designations: a - % of the sample mass, b - % of the total carbon mass, c - % of the total humic acids.*

Horizon depth, cm	Total carbon Corg %	Organic carbon (%) extracted with 0.1M $Na_4P_2O_7$+0.1M NaOH			C_h/C_f	Organic carbon , (%) humic acid fractions		Unextracted Organic carbon (%) (C res.)	Extra. with 0.1N H_2SO_4 (%)	Extracted Corg with 0.1N NaOH %	Soil pH (H_2O)
		total	Humic acids C_h	Fulvic acids C_f		free and bind with R_2O_3	Bind with Ca				
Profile 1. Luvic Gleyic Stagnosol (Aric, Chromic, Clayic)											
Ap 0-26	1.04	0.26[a] 25.00[b]	0.12 11.54	0.14 13.46	0.86	0.00	100.00	0.78 75.00	0.04 3.85	0.20 19.23	4.9
A1 26 - 48	0.90	0.23 25.56	0.09 10.00	0.14 15.56	0.64	0.00	100.00	0.67 74.44	0.04 4.44	0.15 16.17	4.9
Btg1 40-62	0.33	0.08 24.24	0.00	0.08 24.24	-	0.00	0.00	0.25 75.76	0.03 9.09	0.06 18.18	5.0
Btg2 62 – 82	0.27	0.08 29.63	0.00	0.08 29.63	-	0.00	0.00	0.19 70.37	0.02 7.41	0.06 22.22	4.6
Bt gr3 82–108	-	-	-	-	-	-	-	-	-	-	4.7
Profile 2. Dystric Luvic Umbric Planosol (Chromic, Epiclayic)											
AE 0 - 20	1.71	0.74 43.27	0.30 17.54	0.44 25.37	0.68	100.00	0.00	0.97 56.73	0.06 3.51	-	4.4
E 20 – 42	0.45	0.12 46.15	0.08 30.77	0.04 15.38	2.00	100.00	0.00	0.14 53.85	0.04 15.30	--	4.4
Btg1 42-60	0.69	0.17 42.24	0.00	0.17 42.50	-	0.00	0.00	0.23 57.50	0.07 17.5	-	4.6
Btg2 60 – 125	0.45	0.08 30.77	0.04 15.38	0.04 15.38	1.00	0.00	100.00	0.18 69.32	0.04 15.38	-	4.5
BCk 125–150	-	-	-	-	-	-	-	-	-	-	7.4
Profile 3. Epidystric Luvic Albic Planosol (Chromic, Epiclayic, Bathycalcaric)											
AE 0 – 10	0.7	0.34 48.39	0.15 20.77	0.19 27.65	0.75	83.08[c]	16.95	0.36 51.61	0.06 8.33	-	4.5
E1 10 – 28	0.28	0.15 52.20	0.05 18.62	0.09 03.92	0.56	100.00	0.00	0.13 47.80	0.10 37.1	-	4.5
E2 28 - 40	0.18	0.10 53.46	0.04 21.76	0.06 33.70	0.68	13.59	84.41	0.08 46.54	0.09 48.00	-	4.7
Btg1 40-55	0.18	0.09 52.22	0.01 5.00	0.08 44.22	0.1	0.00	100	0.09 47.78	0.08 44.44	-	4.6
Btg2 55 – 73	0.2	0.09 46.76	0.01 4.83	0.08 41.92	0.12	0.00	100	0.11 53.24	0.08 41.63	-	4.8
Btg3 73–96	0.24	0.08 34.92	0.02 9.76	0.06 25.16	0.4	0.00	100	0.18 75.05	0.08 31.96	-	6.6
Ck 96-125	0.15	0.05 33.65	0.03 18.62	0.02 15.03	1.24	0.00	100	0.11 76.35	0.06 37.05	-	8.4

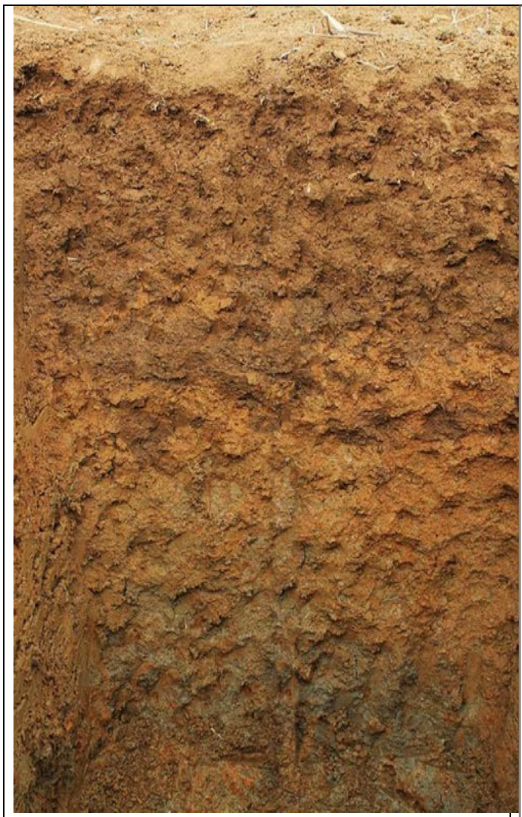
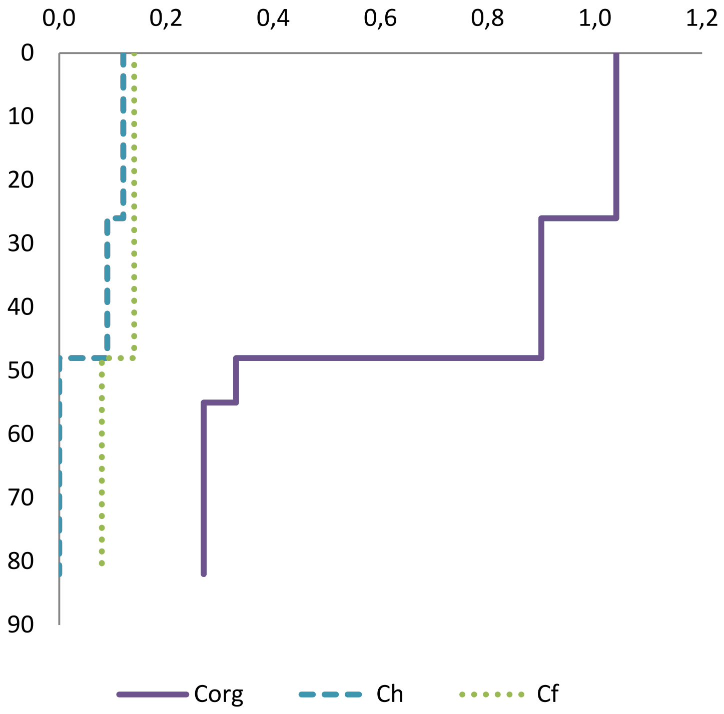


Figure 1. *Organic matter content (% / cm depth) - Profile 1 Picture BG051PO0001-3.3.06-044 project .*



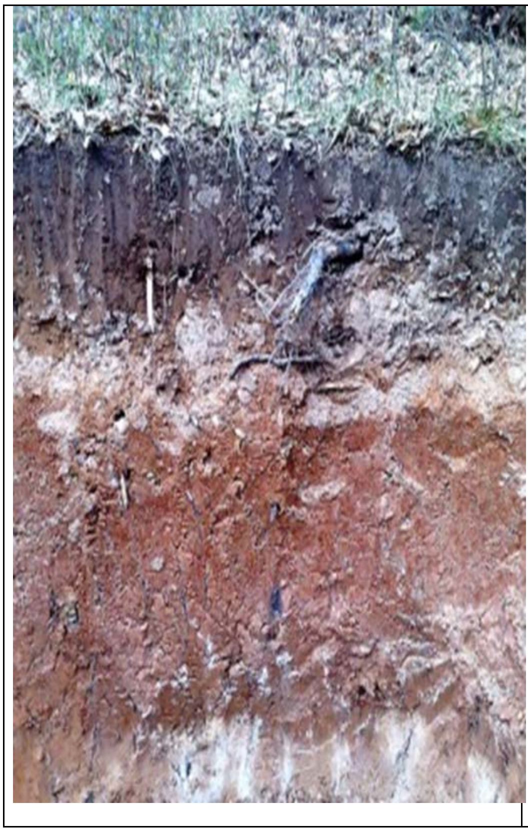
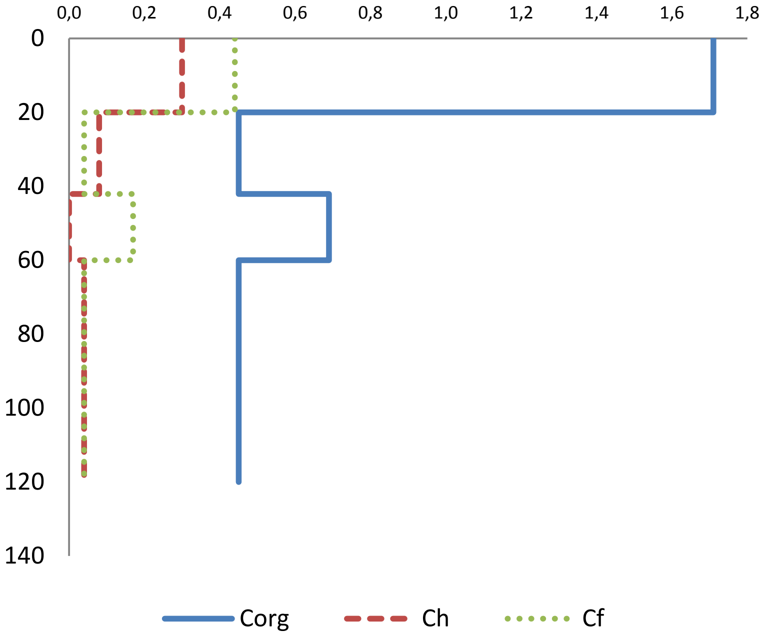


Figure 2. *Organic matter content (% / cm depth) - Profile 2*



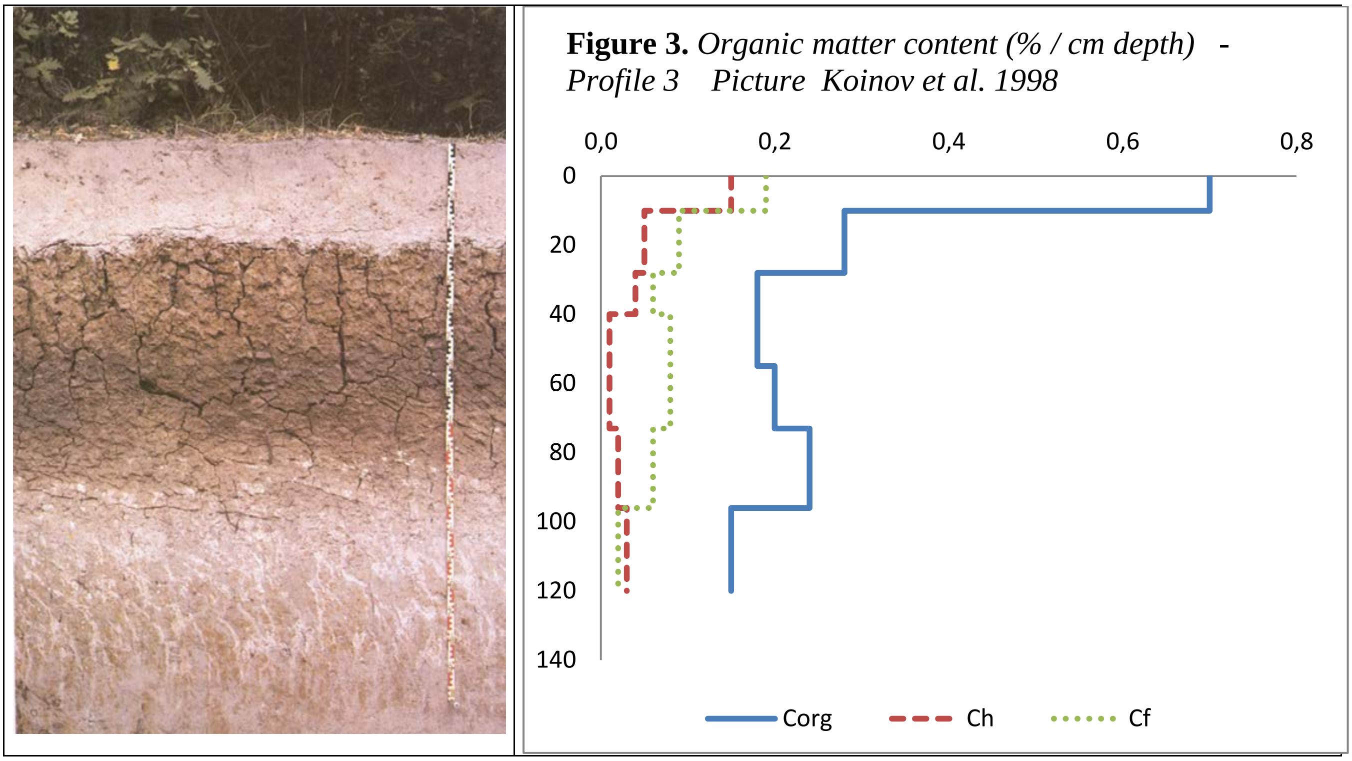


Figure 3. *Organic matter content (% / cm depth) - Profile 3 Picture Koinov et al. 1998*

According to Filcheva (2015), the degree of waterlogging and type of land use (cultivated, uncultivated, forest), indicators of humus state for Planosols are changed. Fulvic acids (C_f) prevail in soil horizons especially in soil surface, because of forest vegetation.

Humic acids (C_h) content is low. They exceed fulvic acids (Ch>Cf) in only three soil horizons in the deeper layers of the profile, where the pH activity is higher. Therefore the type of humus is humic-fulvic (Ch/Cf ratio 0.5 – 1) in surface horizons. In E horizon of profile 2 the type of humus is fulvic - humic (Ch/Cf ratio 1-2) which is not typical for Planoslos. The reason for that could be the influence of clay minerals as smectite and vermiculite which dissociate alkaline calcium and magnesium and humic acids are more stable. In Btg layers of profile 3 the type of SOM is fulvic (Ch/Cf < 0.5), which is typical composition for these stagnic horizons. The degree of humification (Ch/Corg) *100) % in studied soils ranges from average (10-25%) to poor (under 10 %).

Previous studies on soils with stagnic properties show that free humic acids present in surface horizons (Boyadjiev et al. 1994b, 1994c). In profile 1 humic acids are 100 % bound with calcium (Ca-complex), possible reason for that is liming in recent years. In Planosols's surface horizons (AE and E) humic acids are almost free and R_2O_3 complexed. In deeper layers with stagnic properties (Btg) humic acids are 100 % bound with calcium. Consequently humic acids are stable in subsurface horizons. Lack of "free" and bind with mobile forms of R_2O_3 humic acids in Btg horizons may be is due to the influence of acidic vegetation and the possibility to formation of iron-humus complex compound, which could not be extracted with 0.1 N NaOH (Filcheva, 2015).

The unextracted organic carbon in profiles 2 and 3 is averagely about 50 % and it is not so stable. On the other hand unextracted organic carbon in profile 1 is about 75 %, as well as in the deepest horizons in profiles 2 and 3. Therefore the SOM is strongly bound and more stable.

Conclusion

Soils with stagnic properties have low content of organic carbon. Total carbon content decreases sharply in subsurface horizons (Btg). Fulvic acids (C_f) prevail over Humic acids (C_h) in soil profile. The type of humus is humic-fulvic (1-0.5) and fulvic (<0.5). In Planosols's surface horizons (AE and E) the humic acids are almost free and R_2O_3 complexed. In deeper layers with stagnic properties (Btg) humic acids are in minimal quantity and they are totally bound with calcium in profile 1 and 2. The degree of humification in studied soils ranges from average to poor.

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Contemporary Aspects of Soil Eco-Chemistry and Geochemistry in Bulgaria

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Abstract

The paper focuses on the main aspects of contemporary soil eco-chemistry in Bulgaria. The issues tackled refer to: (i) analysis of chemical species, including contaminants; (ii) prediction of metal bioavailability and exposure to plants of chemical species in soil. A stress is made on pilot studies concerning organic geochmical markers, their analysis and quantification in soils (Fluvisols, Vertisols, Luvisols, Arenosols). A new aspect of soil eco-chemistry is related with analysis of compounds in water-soluble dissolved organic carbon in soil as molecular indicators of environmental change.

Key words: soil eco-chemistry, geochemistry, heavy metals, lipids, molecular markers

Introduction

In the early years of soil science, soil chemistry was concerned with agricultural and pedogenetic aspects of soil science, and with chemical characteristics of soil as affected by the mineralogical composition, soil organic matter and environmental factors. With the growing urbanization and industrialization of society, soil chemistry evolved into soil eco-chemistry and "environmental soil chemistry" (Sparks, 2002). Soil eco-chemistry studies the origin, transport, behavior, fate and environmental risk of chemical species in soil and their interaction, including living organisms. It developed as emerging discipline at the intersection of ecology and chemistry of soils. The main aspects of soil eco-chemistry are related with (i) analysis of chemical species, including contaminants; (ii) transport and transformation depending on the soil environment; (ii) ecological and eco-toxicological effects and risk assessment of chemical species; (iii) monitoring of chemical pollutants in the soil-water-plants ecosystems; (iv) prediction and modeling the fate and exposure to chemical pollutants; (v) pollution control. When a contaminant (metallic or organic) is exposed to the soil environment it is subjected to abiotic chemical and/or physico-chemical, but also microbially mediated reactions which may increase or decrease the compound's mobility and bioavailability. These are adsorption/desorption, precipitation, dissolution, complexation and oxidation/reduction reactions.

Assessing the active species of a contaminant in solution will make it possible to predict its fate and toxicity and develop scientifically sound and cost-effective remediation strategies.

State-of-the-art and recent advances

Soil eco-chemistry in Bulgaria has been concerned with basic physico-chemical phenomena: ion-exchange in soils, clays and clay minerals (Arsova, 1998; Atanassova, 1995a,b; Atanassova & Ilieva 2003, Ganev, 1990; Taneva et al. 2010a,b), adsorption/desorption reactions of heavy metals and points of zero charge of soil adsorbents (Atanassova, 1995a; Atanassova 1999; Atanassova and Okazaki, 1997; Atanassova and Benkova, 2013; Atanassova & Simeonova, 2013; Ganev et al., 1993; Jokova, 2005; Jokova et al. 2011), “pools“ of metals in soils (Bacon and Dinev, 2005; Benkova et al., 2005; Chuljian and Hinov, 1976; Dinev, 2012; Faitondjiev and Hinov, 1980), mobility, bioavailability/complexation (Atanassova, 2003; Atanassova and Damyanova 2003a,b; Dinev et al. 2011; Dinev, 2012; Doichinova et al., 2014; Nenova et al., 2015; Raychev et al. 2001; Tsolova et al. 2014). The chemistry of soil solution, the physico-chemical properties of the soil adsorbent, pH, ionic strength and metal speciation in solution affect the mobility, bioavailability and eco-toxicological effects of metals and their sorption to soil particles. Benkova & Atanassova (2015) investigated amelioration of soil acidity in Cu, Zn and Pb contaminated soils around metalliferous industrial plants in Bulgaria, using organo-mineral mixtures containing lime, phosphorite-glauconite, peat, coal and Fe hydroxides. The organo-mineral amendments were effective in increasing the biomass yields and decreasing >70% (Zn) and > 90 % (Cu and Pb) metal solution concentration, as compared to the control variants. However, no significant differences in metals uptake among treated soils and control soils could be discerned, because the major species of Cu, Zn and Pb in the pH range of 5,7 – 7,4 were organic complexes with dissolved organic carbon (DOC) leading to a lack of correlation between solution/plant concentrations. The organic compounds present in the DOC fraction of soils are ligands for complex formation with metals in solution (Fig. 1). These low-molecular-weight organic acids have been identified in the total ion chromatograms of dichloromethane (DCM) fractions of hot-water-soluble extracts in soils (Atanassova et al. 2014, Atanassova & Doerr, 2014, 2015).

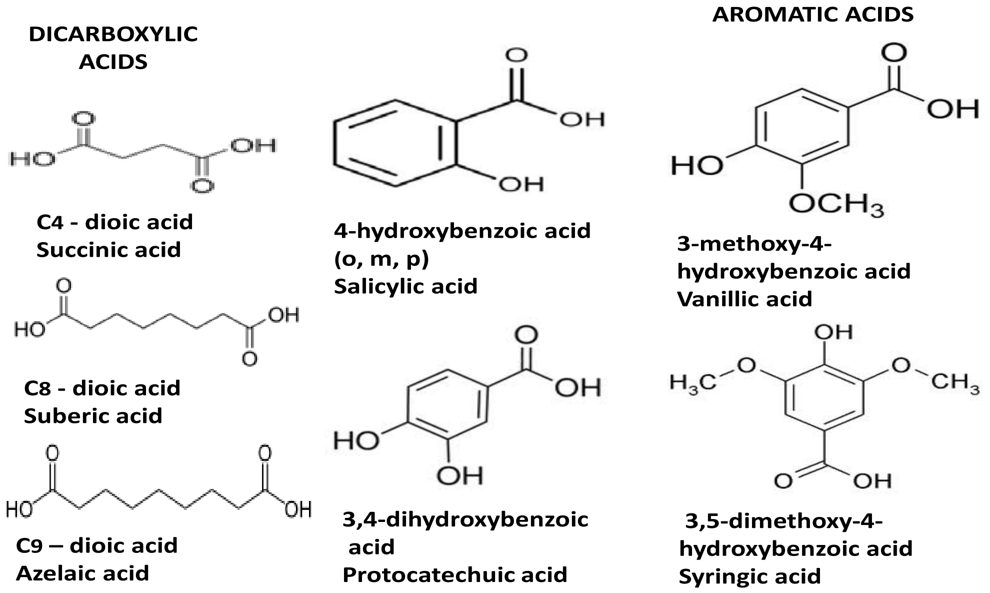


Figure 1. *Low-molecular-weight organic compounds identified in hot-water-soluble extracts of soils (incl. water repellent).*

The geochemical molecular markers are organic compounds *which preserve the signature of their sources and undergo insignificant* structural changes during the process of their transport through the geosorbent. These compounds possess different time of residence, stability, transportation and behavior in relation to their chemical properties and sources (Azevedo, et al., 2002; Yunker et al., 1999). Organic molecular markers are sensitive indicators of ecosystem properties and are related to specific sources of soil organic matter and type of anthropogenic contamination. Through organic marker analysis valuable information on sources in a complex multifunctional system of diverse anthropogenic influences, as well as implications on the transport and fate of these compounds, can be achieved. Molecular markers in organic geochemistry can be subdivided into three major groups as outlined by Eganhouse (1997), namely: (i) contemporary biogenic markers; (ii) fossil biomarkers, and (iii) anthropogenic markers. The first group of markers are compounds that are cellular constituents synthesized by microorganisms and/or vascular plants. Examples are phospholipids, aromatic acids and phospholipid ester bound fatty acids, alkanes, alcohols, chlorophyll and carotenoid containing pigments, high molecular weight terpenoids, etc. The second group consists of compounds detected in fossil fuels and coal deposits formed as a result of thermal alteration of biogenic compounds in a buried biomass deep into the Earth. Some of these compounds, e.g. alkanes, steranes, polycyclic aromatic hydrocarbons resemble in chemical structure the organic compounds synthesized by plants, bacteria and algae. The third group comprises compounds that result from human inputs, e.g. fecal sterols, coprostanol, synthetic surfactants and those directly related to industrial activity, e.g. contaminant assemblages such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAH), chlorinated pesticides, etc. Some PAHs such as naphthalene, perylene and phenanthrene can be detected in tree species and termites (Wilcke, 2000). Thiele and Brummer (2002) find biosynthesis of 3-, 4- cyclic PAH following incubation of fresh plant residues with soil in a reducing environment under abiotic conditions. Other authers also report biogenesis of naphthalene, phenanthrene and benzo(g,h,i)perylene in oxygen-deficient soil environment (Atanassova & Bruemmer, 2004; Guggenberger et al., 1996; Wilcke et al., 1999). The fate and persistence of organic pollutants is a subject of numerous studies (Barraclough et al., 2005, Gevao et al., 2000, Pan et al., 2008). Although organic compounds are gradually degraded and their concentration decreases with time, there is hardly a situation of permanent soil clean-up, due to formation of "bound residues", therefore the widely popular term "persistent organic pollutants" (POPs).

It has been found that only part of the organic compounds extracted by an organic solvent is bioavailable (Luthy et al., 1997). The transport and lability of organic compounds, including pollutants is governed by the following processes: (i) leaching with water flow; (ii) volatilization; (iii) chemical reactions, involving photolysis, hydrolysis, oxidation and reaction with soil organic and inorganic surfaces; (iv) biotic losses including mineralization by microbial action, and (v) soil adsorption. For hydrophobic organic compounds, many of which are classified as "persistent organic pollutants", it has been found that labile forms diminish with time at the expense of tightly bound forms "bound residues" (Jones et al., 1996). According to Lanno (2003), "bioavailability" is a value of the potential of a chemical compound to enter into a biological receptor. The first stage has a physico-chemical trigger and includes adsorption/desorption and diffusion, controlled by the properties of the sorbent and the sorbate, i.e. specific soil parameters, e,g, hydrophobicity, solubility, pK_a, cation exchange capacity, pH, clay and organic matter content. The second stage is physiologically triggered and is controlled by the characteristics of the biological species.

Most of the hydrocarbons have biogenic origin and it's considered that anthropogenic activity actually does not "introduce" hydrocarbons as a new class of compounds into the environment, but has caused their accumulation, and therefore deleterious effects as, e.g. in the case of oil spills (Harayama et. al.,1999). A range of organic compounds, some of which anthropogenic markers induce soil water repellency, e.g. in soils irrigated with treated sewage effluent (Wallach et al. 2005). The classes of organic compounds considered to relate to soil water repellency are alkanes, fatty acids, fatty acid esters, fatty alcohols, sterols and more complex organic compounds (Atanassova & Doerr, 2010; Morley et al., 2005). Recentlly Mataix-Solera et al. (2011) found that the use of low-quality wastewater for disposal purposes, followed by six years of 'recovery' with no irrigation, on a calcareous sandy soil (Xerofluvent) under a *Populus alba* tree stand, led to soil water repellency development. Accelerated solvent extraction of these soils with dichloromethane revealed alkanoic acids (C_{16} - C_{20}), n-alkanols (C_{22}

- C_{28}), steroids of plant and animal origin, and anthropogenic markers, alkylamines and quaternary alkyl ammonium salts (Fig. 2) exhibiting higher concentrations in the water repellent soils in the furrows, as compared to the control non-repellent soils (Fig. 3).

Water repellency appears to have developed where a specific threshold for compound concentration has been exceeded (e.g. n-alkanols ~C_{26}; alkanoic acids $C_{18\text{-}24}$) or for other specific waste water derived compounds, such as sterols (β-sitosterol), cholesterol derivatives, alkylammonium salts or fatty amines/or heterocyclic aromatic compounds (derivatives).

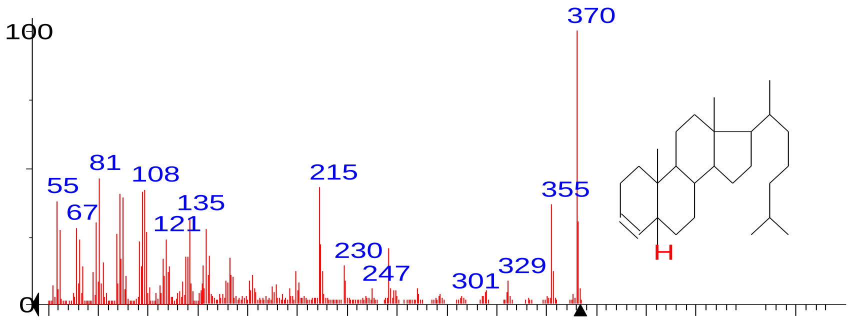
The necessity to make a quantitative assessment of the labile pool of organic carbon in soils arises from changes in the global carbon cycle in response to climate change (Schlesinger and Andrews, 2000), and is of major importance in determining the increase of carbon emissions as a result of anthropogenic activities and global warming. The degree of transformation and mineralization of soil organic matter is a function of climate, vegetation, soil mineral composition, land topography and time. It's considered that temperature is a major trigger determining the cycle of soil organic matter (SOM), especially the rapidly transforming organic fraction (Trumbore, 1997). The climate change effects on the labile fraction of SOM have not been studied in detail (Franzluebbers et al., 2001). Unravelling the feedback effect to climate change is particularly difficult, because of a range of diverse soil organic compounds exhibiting different decomposition kinetics and the presence of environmental constraints which obscure the intrinsic temperature sensitivity of substrate decomposition (Davidson and Janssens, 2006; Schmidt et al. (2011).

The labile and bound lipids and the lignin originating phenolic compounds are an important component of SOM (Dinel et al., 1990; Kögel-Knabner, 2000). The free lipids from plant waxes and the biopolymers cutin and suberin are representative of the aliphatic component of SOM (Kögel-Knabner et al., 1992; Kögel-Knabner, 2000). The content and composition of the free lipids of SOM depends on the specific vegetation and the soil physico-chemical properties (van Bergen et al., 1997; Dinel et al., 1990).

Organic geochemical studies with soils, clays and sediments in Bulgaria have been concerned with introducing innovative methods of analysis of organic compounds (geochemical markers) in natural and degraded soils (water repellent, anthropogenically and technogenically affected), (Atanassova and Doerr, 2010, 2011; Atanassova and Doerr, 2014, 2015; Atanassova and Teoharov, 2010; Atanassova et al. (2012); Atanassova et al. (2014). Organic contaminants, including persistent organic pollutants (POPs), their derivatives and emerging organic pollutants in soils, clays and sediments have received attention in the studies of Atanassova and Brümmer (2004); Atanassova and Teoharov (2010); Atanassova et al., (2014, 2015); Atanassova and Mills (2016).

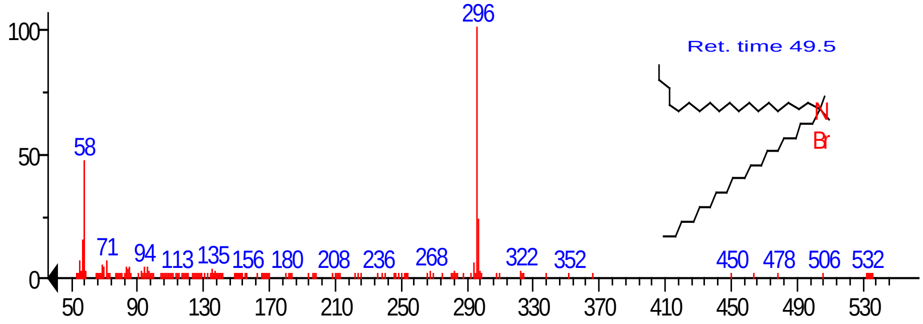
The advances in organic geochemical marker analysis are related to both methodological and fundamental aspects of identification, quantification and source apportionment. New methods of analysis have been elaborated and applied in various geosorbents: soils, sediments and clays. Analysis has been carried out by making use of accelerated solvent extraction (ASE), Soxtec and sonication (Atanassova and Doerr, 2010, 2011, Atanassova et al., 2012, Atanassova and Teoharov, 2010, Atanassova et al. 2014). Recently, Atanassova and Doerr (2015) using alkaline saponification of dichloromethane (DCM) and dichloromethane/iso-propanol (DCM/IPA) fractions of hot-water-soluble extracts, have identified esterified aromatic acids contributing to soil water repellency in water repellent soils.

Compound 36



(mainlib) Cholest-3-ene, (5á)-

Compound 52



(mainlib) Dimethyldioctadecylammonium bromide

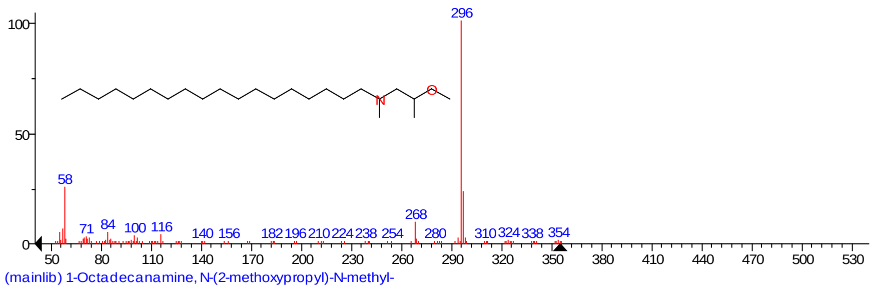


Figure 2. *Detected anthropogenic markers in a waste water treated soil (Xerofluvent) from Alicante, Spain.*

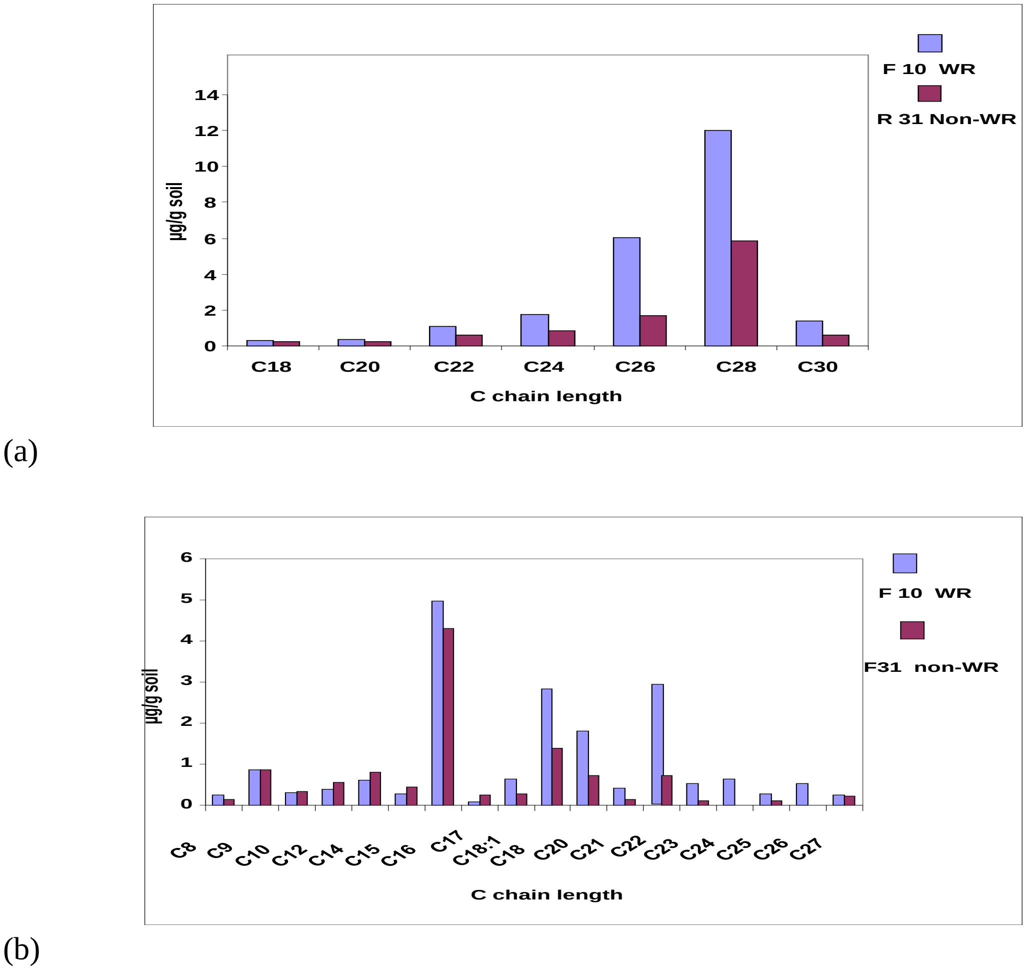


Figure 3. *a) Absolute concentrations of n-alkanols in water repellent soils (furrows F10) and non-water repellent soils (ridge F31); b) Absolute concentrations of n-fatty acids in water repellent soils (furrows F10) and non-water repellent soils (ridge F31).*

Case studies

In a recent study, Atanassova and co-workers from the Institute of Soil Science, Agrotechnologies and Plant Protection (ISSAPP) in Sofia have investigated anthropogenically affected soils from the South-West of Kremikovtsi village. Analyzed were the signatures of lipid biomarkers in a Cinnamonic meadow soil (*Rhodic Gleyic Luvisol*) under the addition of 4,2 t/dka sludge from the Sofia Waste Water Treatment plant (WWTP) and from the experimental field at Bojurishte (*Vertisol*) under the effects of various conventional and organic farming, addition of green manure and withering of plant residues, as well as animal manure.

Long chain alkanes were detected with predominance of odd-over-even homologues maximizing at C_{31}, which shows a similar signature with that of genera *Hedera* (ivy), *Rubus* (blackberry, van Bergen et al. 1997), pine needles (Jambu et al. 1991), *Fraxinus*, grass species (van Bergen et al. 1997) and grass roots (Marseille et al. 1999). Alkane distribution (Fig. 4-5) shows dominance of vascular plants and a similar signature to that found in grazing fields and stubbles (van Bergen et al. 1997). The alkane distribution pattern is similar in the soils from Bojurishte following incorporation of maize/rye and maize/peas residues. The range of long chain alkanes with a maximum at C_{31} resembles that in soils with manure addition and follows unimodal distribution in the range C_{20} - C_{33} maximizing at C_{31}. Fatty alcohols distribution is also unimodal in the range of C_{22} - C_{32} maximizing at C_{28}. Relative amounts of the *n*-alkanols are the following: C_{22} – 13%, C_{26} – 11,2%, C_{28} – 24,3%, C_{30} – 13%, C_{28} – 24,3, C_{30} – 13%, C_{32} – 3,7. In the soils treated with farmyard manure C_{24}, C_{27}, C_{28}, C_{29}, and C_{32} fatty alcohols were detected. The high proportion of the *n*-alkanols in the soils treated with farmyard manure (FYM) confirms the results of Bull et al. (1998). Very low intensities of fatty acids were obtained, most possibly due to a strong adsorption in the clay fraction of the Vertisol. Other identified compounds were plant sterols: stigmasterol, β-sitosterol, stigmast-4-en-3-one, C_{18} fatty acid, 2-[(1-oxotetradecyl)oxy]-1,3-propanediyl ester.

In the deeper soil horizons of the meadow Cinnamonic soil from the region of Kremikovtsi a different n-alkanes and n-alkanols distribution has been found with a maximum at C_{24} for n-alkanols instead of C_{28} in the range of C_{16}-C_{30} (Fig. 6-9), showing the increasing role of the microbial source at the lower depth. The alkane distribution shows a CPI index ~ 1, which again implies a microbial source of soil organic matter.

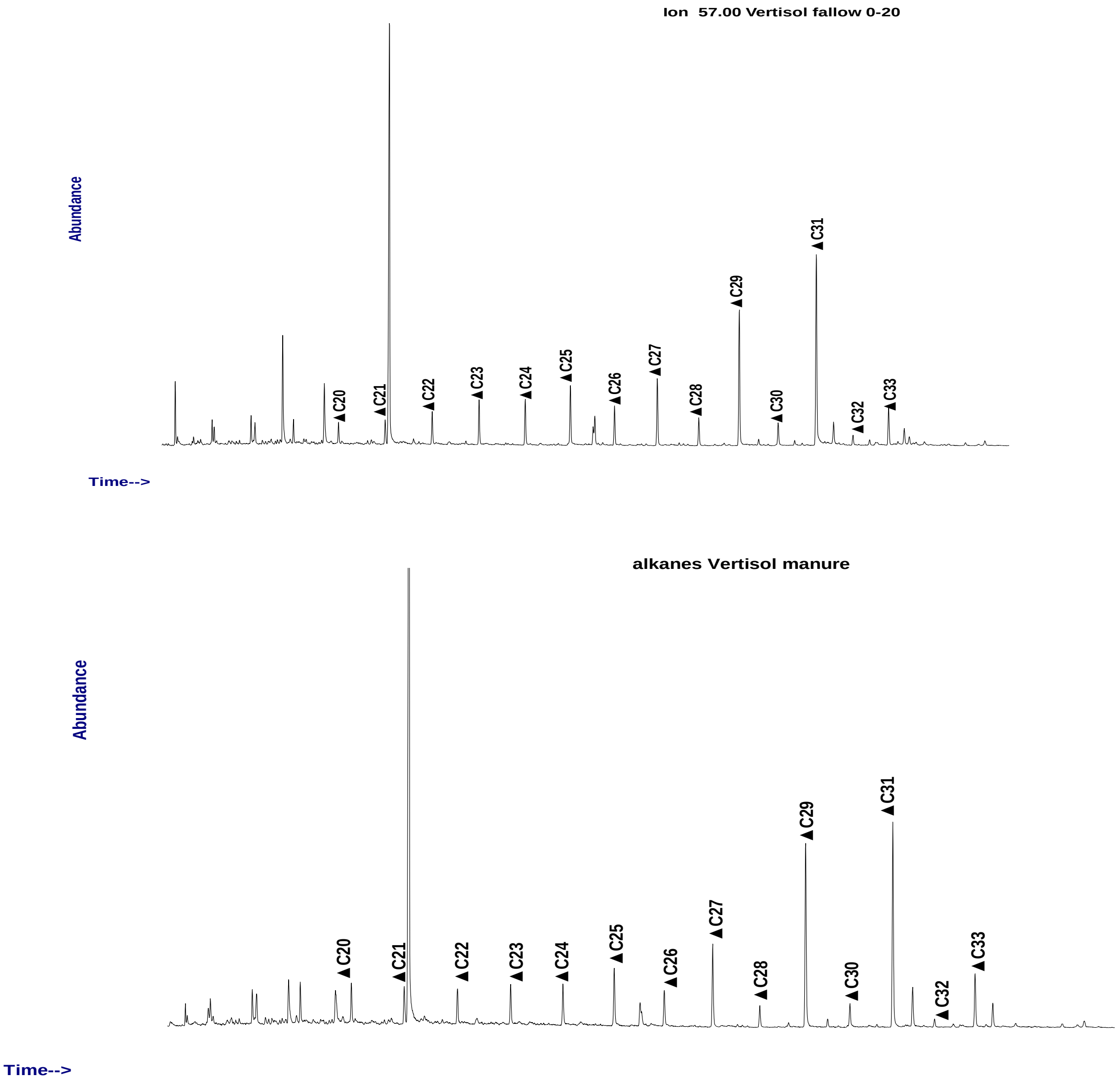


Figure 4. *Distribution of alkanes, following addition of manure in a soil (Vertisol) from Bojurishte.*

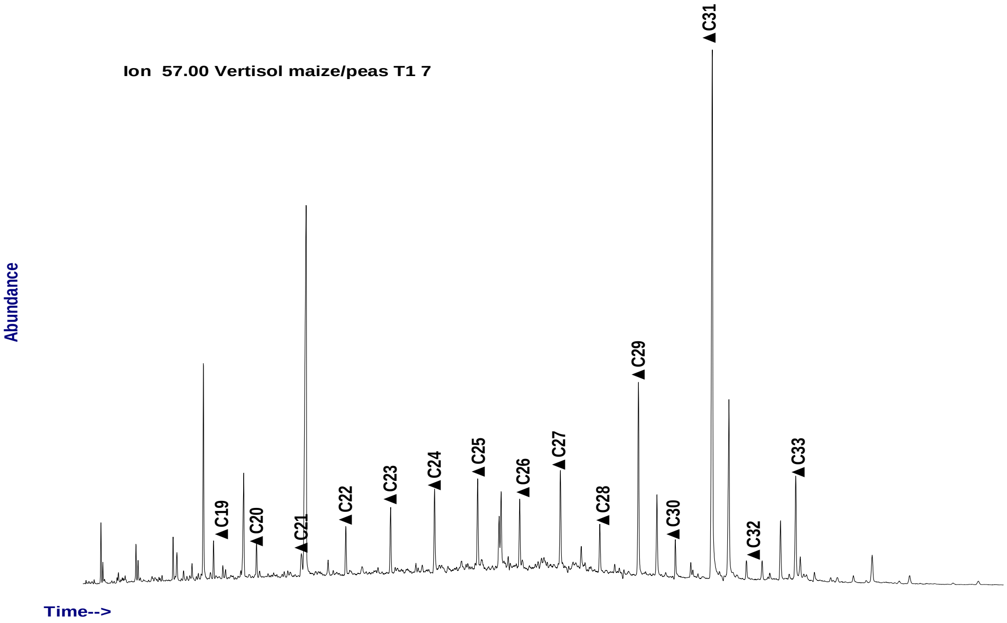


Figure 5. *Distribution of n-alkanes (m/z 57) in a Vertisol under green manure (maize/peas).*

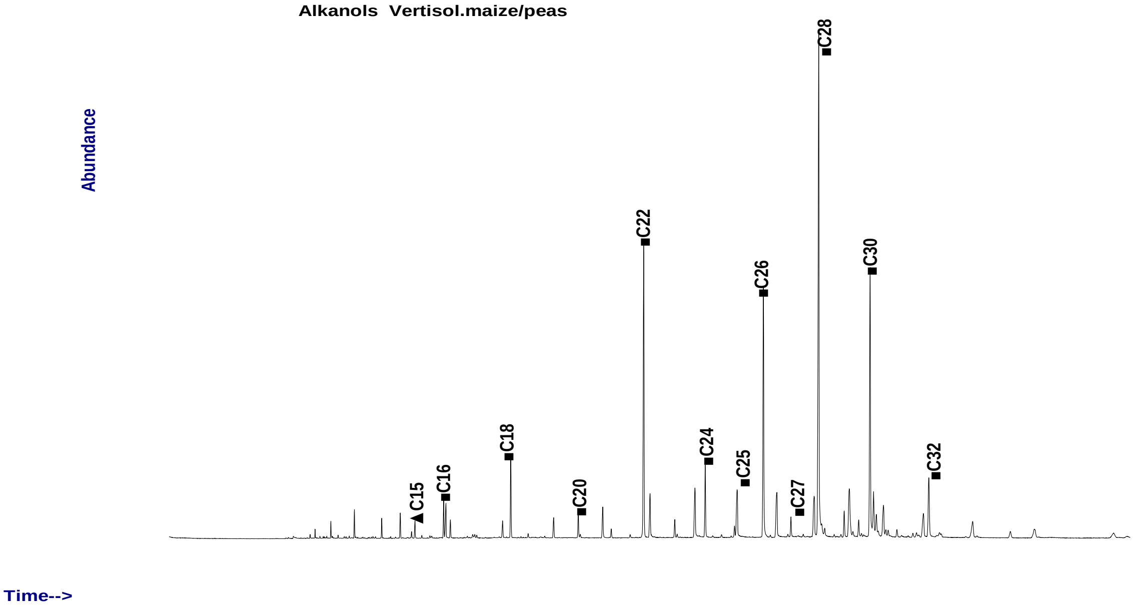


Figure 6. *Distribution of n-alkanols (ion m/z 75) in a Vertisol under green manure maize/peas.*

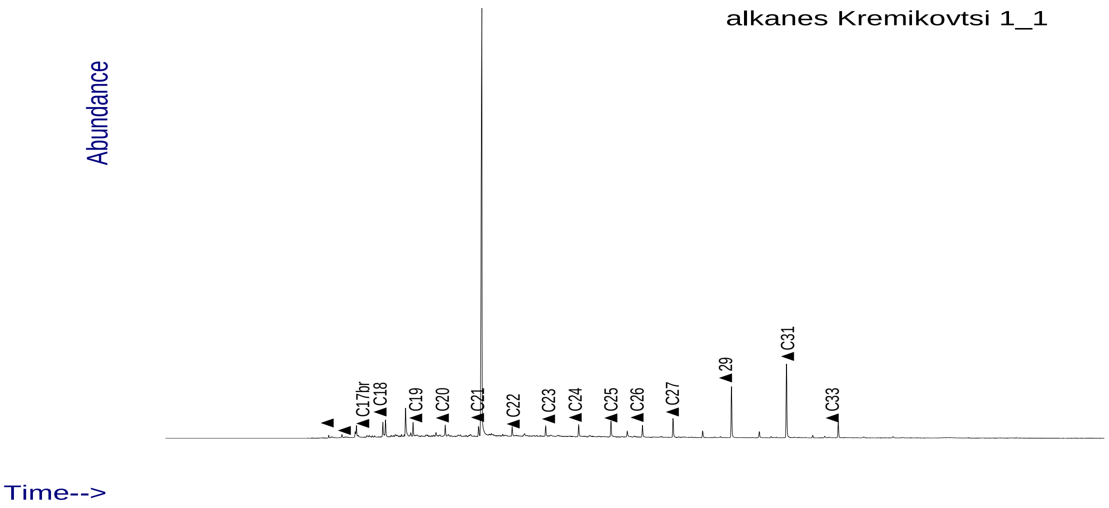


Figure 7. *Distribution of n-alkanes (ion m/z 57) in a meadow Cinnamonic soil under sludge.*

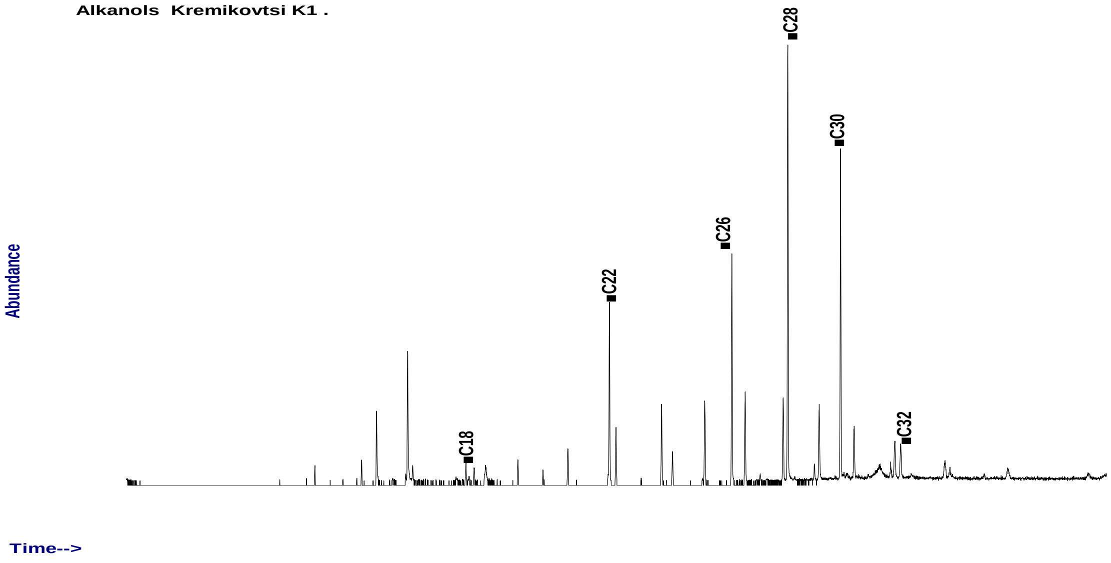


Figure 8. *Distribution of n-alkanols (m/z 75) in a meadow Cinnamonic soil under sludge from Sofia WWTP.*

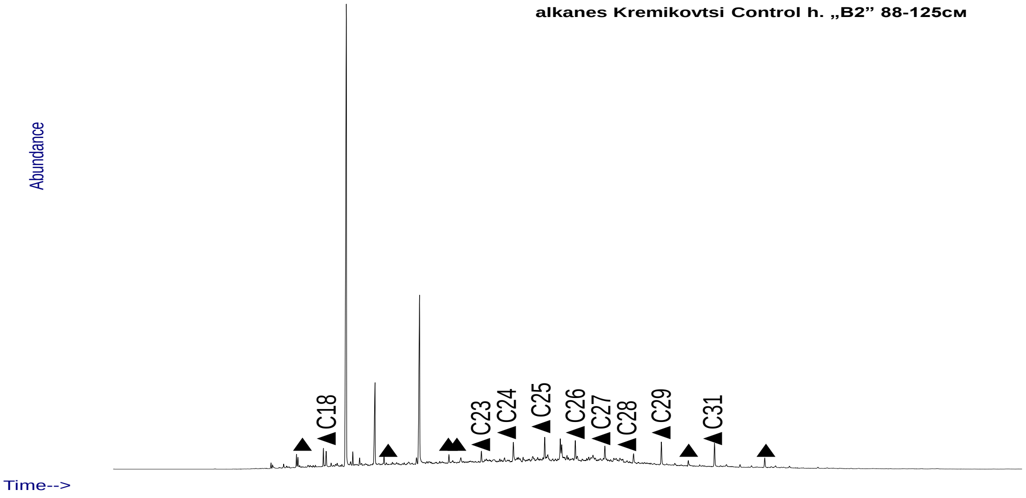
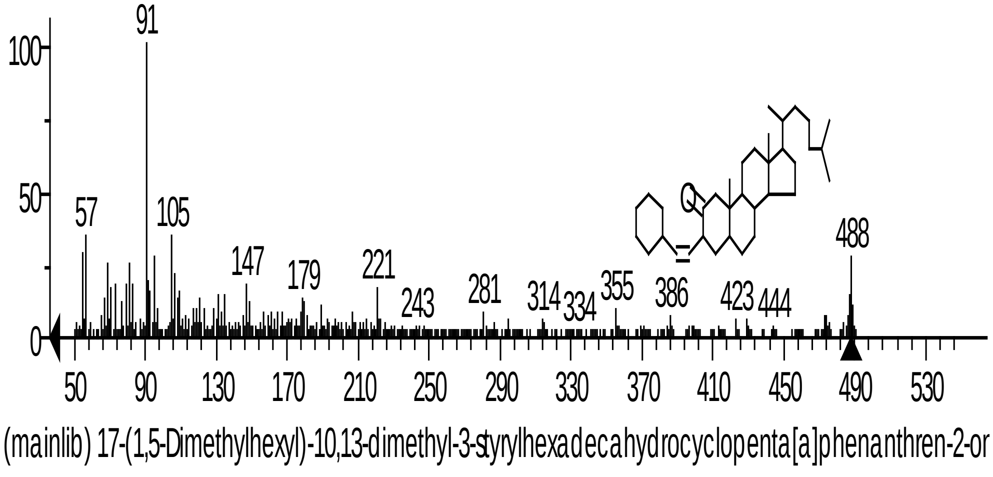


Figure 9. *n-Alkane distribution in Kremikovtsi soil (untreated).*

In a Meadow Cinnamonic soil (Urbic Garbic Mollic Technosols, Calcaric Humic Siltic) under deposited sludge from paint and print industry (Atanassova et al. 2011) some characteristic compounds in municipal sewage sludge were detected such as: 17-(1,5-Dimethylhexyl)-10,13-dimethyl-3styrylhexadecahydrocyclopenta[a]phenanthren-2-one; 3-acetoxy 7,8-epoxylanostan-11-ol (detected in oil spills), (Fig. 10); cholest-23-ene, (Z)- ; androstane.



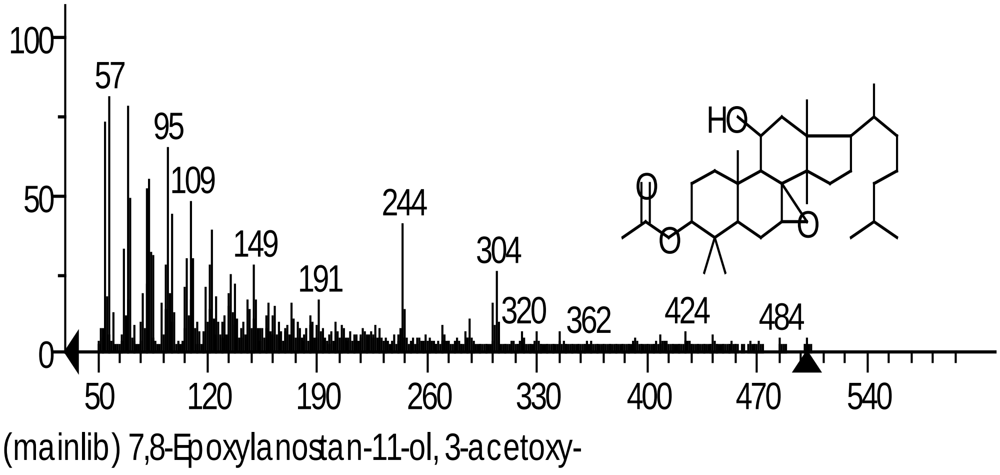


Figure 10. *Anthropogenic markers in sewage sludge from paint and print industry.*

In the B_{u2}–B_{gk} technogenically affected horizons, the following contaminants were detected: exo-tricyclo[5.2.1.0(2.6)]decane, a component in pulse-detonation engines is likely a contaminant compound. Another compound was 2-methyl-4-keto-2-hydroxy pentane released to the environment as a result of its use as a solvent, additive or synthetic intermediate for many materials.

In the gleyic G_{or} horizon, a suspected contaminant isopropyl myristate has been detected in the upper B_{gk} horizon. In addition, a compound with MF 62 similar to acenaphthylene, 1,2-dihydroxy with characteristic fragment ions m/z 55, 73, 262, 328 was detected in B_2 and B_{tg} horizons, but not in G_{or} horizon (Atanassova et al., 2015).

Future challenges and trends in soil eco-chemistry

The future challenges and developments of soil eco-chemistry will have to answer the following critical questions: (i) What is the structure and chemical (physico-chemical) behavior of persistent organic pollutants and their derivatives (metabolites) in soil? How does compound's mobility and toxicity to organisms change in relation to soil environment? (ii) What is the fate of nanoparticles in soils depending on the size, shape, surface charge and soil characteristics, e.g., pH, ionic strength, organic matter, and clay content? (iii) What decision-support systems and remediation techniques should be elaborated to cope with mixed contamination in soils? (iv) Does metal toxicity stop the biodegradation of POPs? (v) What are the living receptors of toxic compounds in the environment?

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HEAVY METALS IMPACT ON SEDIMENT MICROBIAL COMMUNITIES IN RIVER-DAM SEQUENCE OF SMALL HYDROPOWER PLANT CASCADE

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Abstract

Heavy metals are widespread pollutants with significant environment risk due to high toxicity and clear tendency for accumulation in different matrices – soils, sediments, biota. The main research objective of this work is to assess the impact of heavy metal pollution on key structural and functional parameters of microbial communities in sediments of river-dam sequence of small hydropower cascade Middle Iskar, Bulgaria. The content of heavy metals (As, Cd, Cu, Hg, Pb, Zn) was measured during the low water summer periods of 2012, 2013 and 2014. The evaluation of site quality and heavy metal pollution was done by use of one integrated index - Pollution Load Index and it was compared to total count of sediment microbiota and count of coliform bacteria, also with total dehydrogenase activity and index of phosphatase activity. The assessment of heavy metal pollution in river-dam sequence of cascade indicates the higher metal concentrations and high Pollution Load Index in dam sediments. At low level of pollution in river sites, the both structural and functional microbial parameters react to local variations of heavy metal concentrations and high negative correlation (r=-0.8÷-0.9) exists between variables. But in dam sites, the microbial community is more resistant to pollution and structural parameters react conservatively with long reaction time. The enzyme activities are more adaptive and sensitive indicators for different level of environmental impact in this case. The complex phosphatase and dehydrogenase activities have a high potential to be used as reliable parameters for precise assessment of hazardous sediment pollution in complicated ecological situations with cumulative impacts.

Keywords: heavy metals, sediments, microbial community, dehydrogenases, phosphatases, small hydropower cascade

Introduction

Sediments are one of the most diverse habitats in freshwaters and have a specific function of sink and source for organics, nutrients and specific pollutants in ecosystems (Torsvik et al., 2002; Santos et al., 2003; Zhu et al., 2012). Sediment biota, especially microbial communities regulates and affects many ecosystem processes in freshwater sediments and is the main contributor for the transformations of different accumulated compounds (Nealson, 1997; Yu et al., 2015). But the hazardous pollutants with high absorbance and retention capacity are considered as serious threat for sediment status, biotic processes and ecological balance due to their high toxicity, persistence and non-degradability in the environment (Pekey, 2006; Sakan et al., 2015; Tang et al., 2014). Heavy metals are classic examples for this type of sediment-associated hazardous pollutants with high risk level, prevalence and serious impact on biota. The high concentrations of heavy metals are not only toxic for alive organisms but they inactivate many of key transformation processes, damage the ecosystem metabolism and functional integrity (Khan et al., 2007; Todorova and Topalova, 2013; Todorova et al., 2015).

The evaluation of heavy metal pollution status of river bed sediments and pollutants interaction with key biotic complexes is a multiplex task with a series of difficulties in its application. The difficulties are complicated additionally from alterations in hydromorphology and disturbance of normal hydrological regime of natural ecosystems – standard consequence from energy utilization of water. The small hydropower plants (SHP) are considered as environmentally sound alternative of fossil fuels but the construction of cascade of several SHPs strongly affects the degree and rate of sedimentation and pollutants accumulation in alternating “river-dam” sequence (ASTAE, 2014). Cascade exploitation dramatically disturbs the balance of sediment inflow and outflow, increases flow depth, decreases flow velocity and causes intensive settling in dam sites (Sumi and Hirose, 2009). The sediment-related problems such as heavy metals pollution are multiplied and intensified since this additional sedimentation and dams are considered as serious pollutants traps. Accumulation of heavy metals magnifies their negative impact on all aquatic organisms and ecosystem processes, including microbial communities and their enzyme activities (Baby et al., 2010). This influences the self-purification processes in the sediments as well as the ecosystem health.

The aim of this work is to assess the interaction between heavy metals pollution and sediment microbial communities (as total count of indicator groups and activities of some key enzymes) in river-dam sequence of SHPs cascade “Middle Iskar”. The first element – heavy metals impact on biota is well known and many surveys are published (Yu et la., 2015; Baby et al., 2010; Rial et al., 2011; Kapoor et al., 2015; Neethu et al., 2015). But the study of this impact from the view of cumulative effect of alternating “river-dam” sequence is not well-developped and the gap in management plans of this type modified ecosystems is significant.

Materials and Methods

Study area

The study area is localized in the catchment of Iskar River, Bulgaria - 33 km sector of SHPs cascade “Middle Iskar” between Rebrovo and Gabrovnitza villages (Fig. 1). The Iskar River is situated in the NW part of Bulgaria and belongs to the Danube River Basin. Iskar is the longest

Bulgarian river (368 km) with 8 650 km² basin area and mean annual runoff of 37.57 m^3/s in the study sector (Todorova et al., 2015). The river seasonal flow is typical for continental climate zone with a low run off in summer and winter and a high run off in spring and autumn. In its middle part the river flows through a densely populated and industrialized region and is a long-term receptor of treated or non-treated urban and industrial sewage of Sofia area. The river sector is a subject of intensive hydromorphological impact, too - the construction of Middle Iskar Cascade with barrages and power stations has been started since 2000s and now 5 SHPs are in operation.

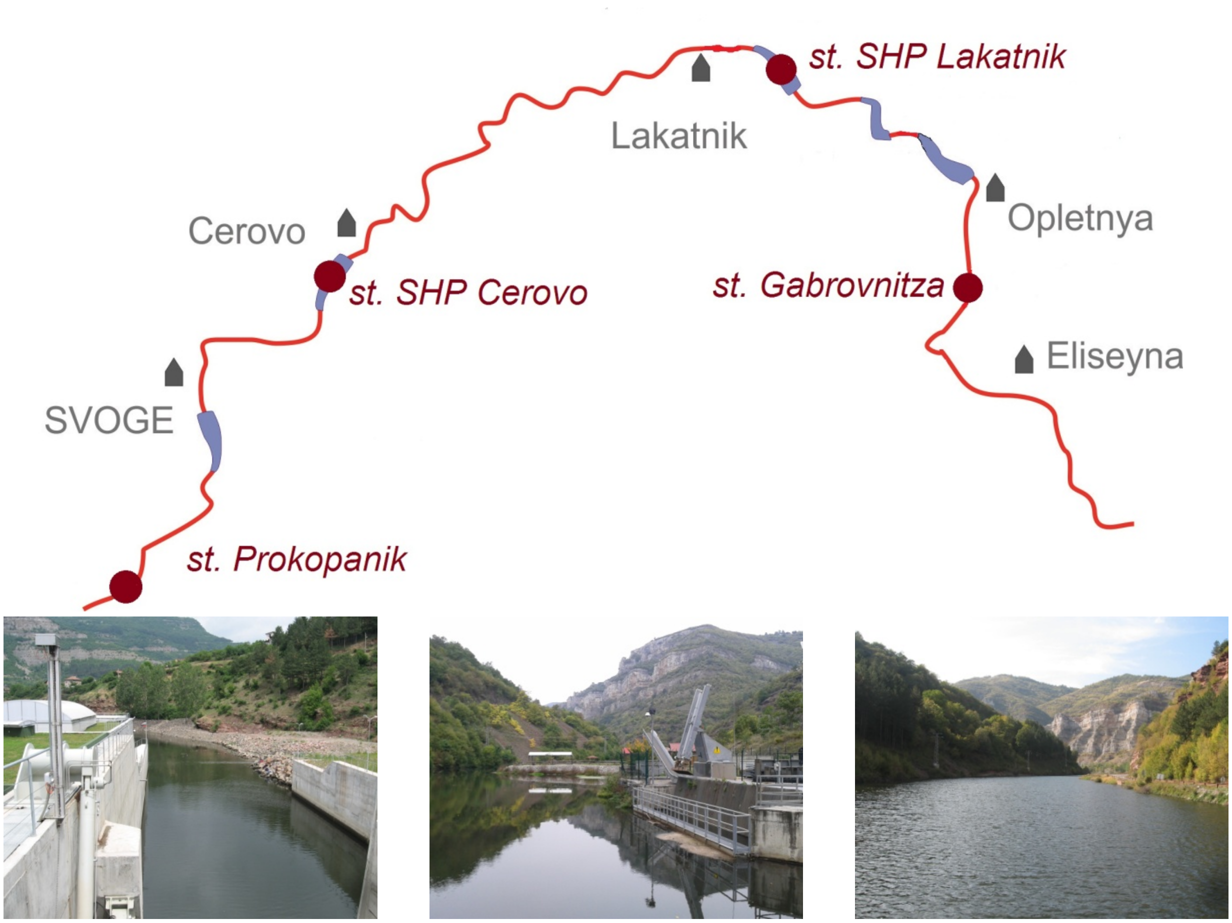

Fig. 1. *Scheme of study area with sampling sites location and pictures of SHPs cascade*

Experimental design and sampling procedure

The present study was conducted during the low water summer periods of 2012, 2013 and 2014 in four sampling sites. The first site was at the beginning of the river sector – Prokopanik (Prok); the second and third sites were at dam walls of SHP Cerovo (Cer) and Lakatnik (Lak); the last was at the end of river sector Gabrovnitza (Gabr) (Fig. 1). The hydrological regime of river sector is strongly modified from the cascade and the study sites can be subdivided into two hydrologically different habitats: "river" sites (Prok and Gabr) and "dam" site (Cer and Lak). In two types of habitats, the content of heavy metals (As, Cd, Cu, Hg, Pb, Zn) was measured; the assessment of site quality and heavy metal pollution was done by use of one integrated index - Pollution Load Index and it was compared to key structural and functional parameters of sediment microbial community.

The sediment samples were collected using Eckman-Berge dredge with an approximated area of 0.04 m^2 for "dam" sediments and by manual dredging for "river" sediments. In site, the stones and plant fragments were removed by passing the samples through a 2 mm sieve. Each collected sample for analyses of heavy metals consisted of at least 1 kg of sediment and was contained in a separate sealed plastic bag. To assess quantitative and functional parameters of sediment microbial communities, individual samples were collected in sterile containers. All of the samples were placed in a cooler at 4◦C and transported to the laboratory immediately. The environmental conditions at the sampling stations were also recorded.

Analytical procedures

For analyses of heavy metals content the sediment samples were air dried, then powdered and finally passed through a 500 µm sieve. The concentrations were determined by atomic absorption spectrophotometry for As, Cd, Cu, Pb and Zn and by cold vapour atomic absorption spectrometry for Hg. The results were presented in mg/kg dry sediments.

Sediment microbial parameters – Total Microbial Count (TMC) and number of coliforms (Colif) were determined by use of count-plate technique on Nutrient agar and Endo-agar for 24-48 h at 35ºC. For enzyme assays the sediment samples were sonicated (UD-20 automatic) 3 x 10 s. Dehydrogenase activity (TDA) was measured according to the method of Lenhard et al., 1964 by the reduction of 2,3,5-triphenyl tetrazolium chloride. Phosphatase activity index (PAI) was determined as an average value of activities of acid, neutral and alkaline phosphatases. The method was based on transformation of p-nitrophenolphosphate (Matavulj et al., 1990). The enzyme activities were presented per mg of total sediment protein. The protein content was determined according to micro-biuret method (Kochetov, 1980).

Calculation of PLI (Pollution Load Index)

For assessment of heavy metals contamination status in sediments of Middle Iskar Cascade, the Pollution Load Index (PLI) was calculated following the method proposed by Tomlinson et al., 1980. This empirical index is a simple, comparative way for assessing the level of heavy metal pollution (Kalender and Çicek Uçar, 2013). This pollution index with simple numeric interpretation is a powerful tool for processing, analyzing, and conveying raw environmental information to decision makers, managers, technicians, and the public (Caeiro et al., 2005). The PLI is based on the contamination factor (CF) of each metal (Hakanson, 1980). The CF is the ratio obtained by dividing the concentration of each metal (C_{sample}) in the sediment by the background value ($C_{background}$ - geochemical background concentration of the metal in Iskar River catchment according Cholakova, 2004) (1). PLI was calculated as the nth root of the product of the n CF (2). Values of PLI<1 or =1 indicate heavy metal loads close to the background level, and values above 1 indicate pollution.

$$CF = C_{sample}/C_{background} \qquad (1)$$

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)} \qquad (2)$$

Data interpretation and statistical analyses

The presented data were means of three or more repeats. A Pearson correlation with statistical significance set at $p < 0.05$ was used to confirm the relationships between microbial parameters and Pollution Load Index. Statistical analyses were performed using Microsoft Excel 2000/XLSTAT (2014.5.03, Addinsoft, Inc., Brooklyn, NY, USA).

Results and Discussion

Heavy metal content in sediment

Figure 3 presents the results for concentrations of heavy metals in sediments of 4 sampling sites. The data for heavy metal concentrations was interpreted according to local quality standards for soils (Regulation No3, 2008) due to the ongoing procedure for development of the sediment environmental quality standards. The metals content varies between: 4-33 mg/kg for As, 0.2-3 mg/kg for Cd, 39-120 mg/kg for Cu, 0.05-0.44 mg/kg for Hg, 25-110 mg/kg for Pb and 132-441 mg/kg for Zn. Despite that the comparison with previous studied periods (1991-2001) shows the decrease in total metal content in river subcatchment (Cholakova, 2004), some of the metals continue to exceed the standards and this specific type of pollution is still a serious risk for the ecosystem. The exceeding with 10-50% of maximum admissible concentrations was registered for As in 2014 at Prok., for Cd and Zn at dam sites. As a trend, the higher concentrations of heavy metals, excepting the arsenic were detected in the sediments of dam sites – Cerovo and Lakatnik. The alternating conditions of running-standing waters are favorable factor for additional increase of heavy metals concentrations in the impoundments of cascade. The arsenic had a different behavior and increased its content in river sites - high concentration of 33 mg/kg was measured in 2014 at Prokopanik sediments.

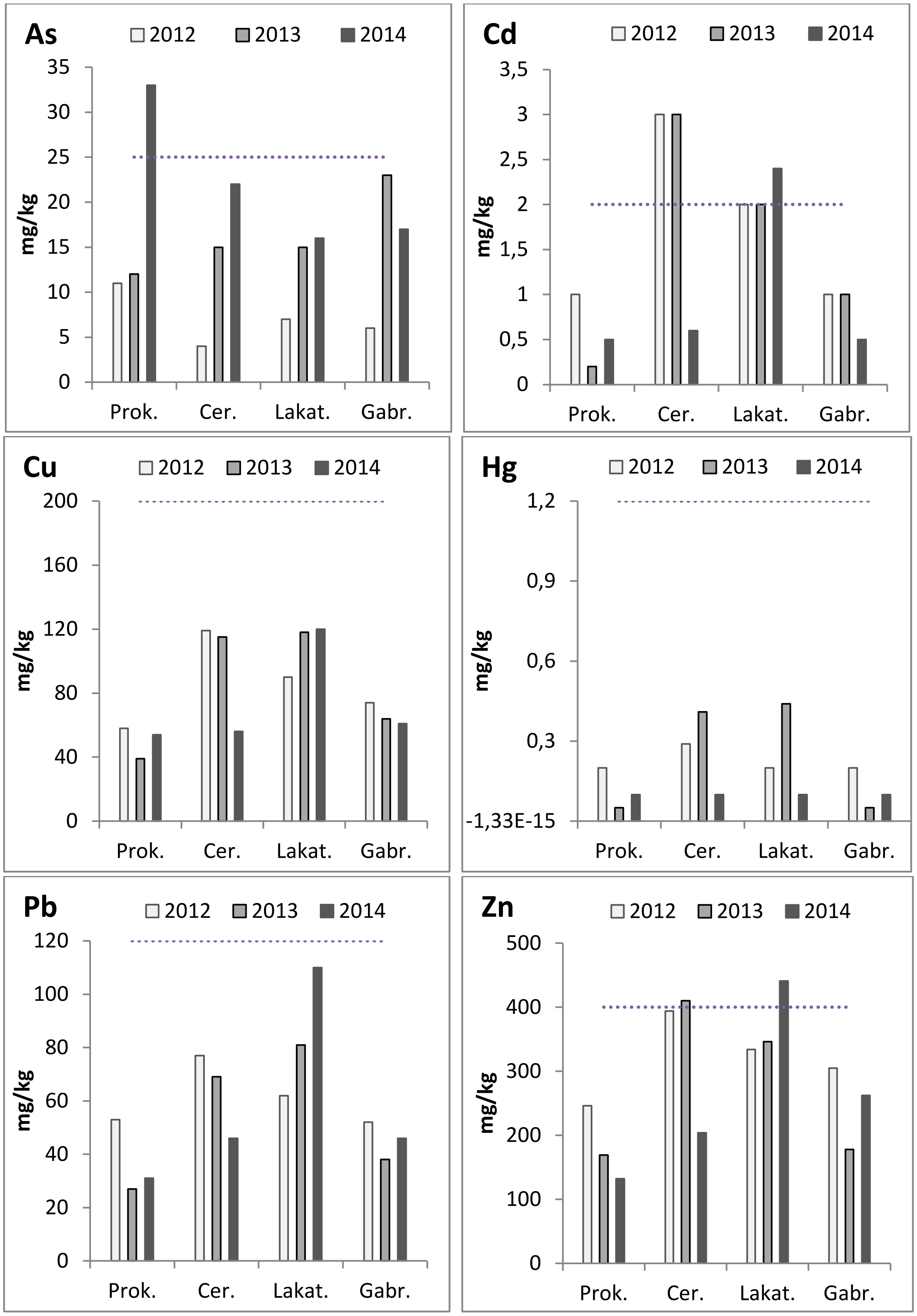

Fig. 2. *Content of heavy metals in sediments of cascade during 2012-2014*

** the dot line is maximum admissible concentrations for each metal according quality standards for soils (Regulation No3, 2008)*

Pollution Load Index (PLI)

The results for Pollution Load Index are summarized in Table 1. The PLI provides simple but comparative means for assessing a site quality and heavy metals pollution, where a value of PLI < 1 denotes good ecological situation; PLI = 1 presents that only baseline levels of pollutants are presented and PLI > 1 would indicate deterioration of site quality and presence of pollution (Tomlinson et al., 1980). The index analyses showed that the pollution was detected in dam sites of Middle Iskar cascade – values ranged between 1.14÷1.72. Although there is no pollution in river sediments, the index has increased values in the last sampling site – indicator for negative role of SHPs cascade in retention of this type of pollutants.

Table 1. *The PLI (Pollution Load Index) values in the sampling sites (values >1 indicate heavy metal pollution)*

	Prok	Cer	Lak	Gabr
PLI_{2012}	0.942	**1.327**	**1.147**	0.948
PLI_{2013}	0.459	**1.723**	**1.634**	0.775
PLI_{2014}	0.736	0.795	**1.461**	0.805

Sediment microbial parameters and heavy metal impact

The PLI is used to present the integrated heavy metal impact of some structural and functional parameters of sediment microbial community. The selected microbial parameters are total counts of microorganisms and coliforms, and total activities of dehydrogenases and phosphatases. The effect of heavy metals pollution on these microbial parameters in river habitats is presented on Fig. 3. The results show a clear negative impact of high heavy metals content despite the PLI indicates “no pollution” in these sites. The high values of index close to 1 are related to low counts of microbial indicators and low enzyme activities. The inhibition of coliforms and total dehydrogenase activity was more expressed – 10 and 3-fold decrease was registered at Prokopanik in 2012 compared to 2013 when the four studied microbial parameters had the highest values at the lowest index of 0.46. The same trend was observed at other river site Gabrovnitza. Correlation analyses confirm the strong relationships between studied microbial parameters and pollution index – high negative correlation with coefficients -0.8÷-0.9 exist between variables in river sites.

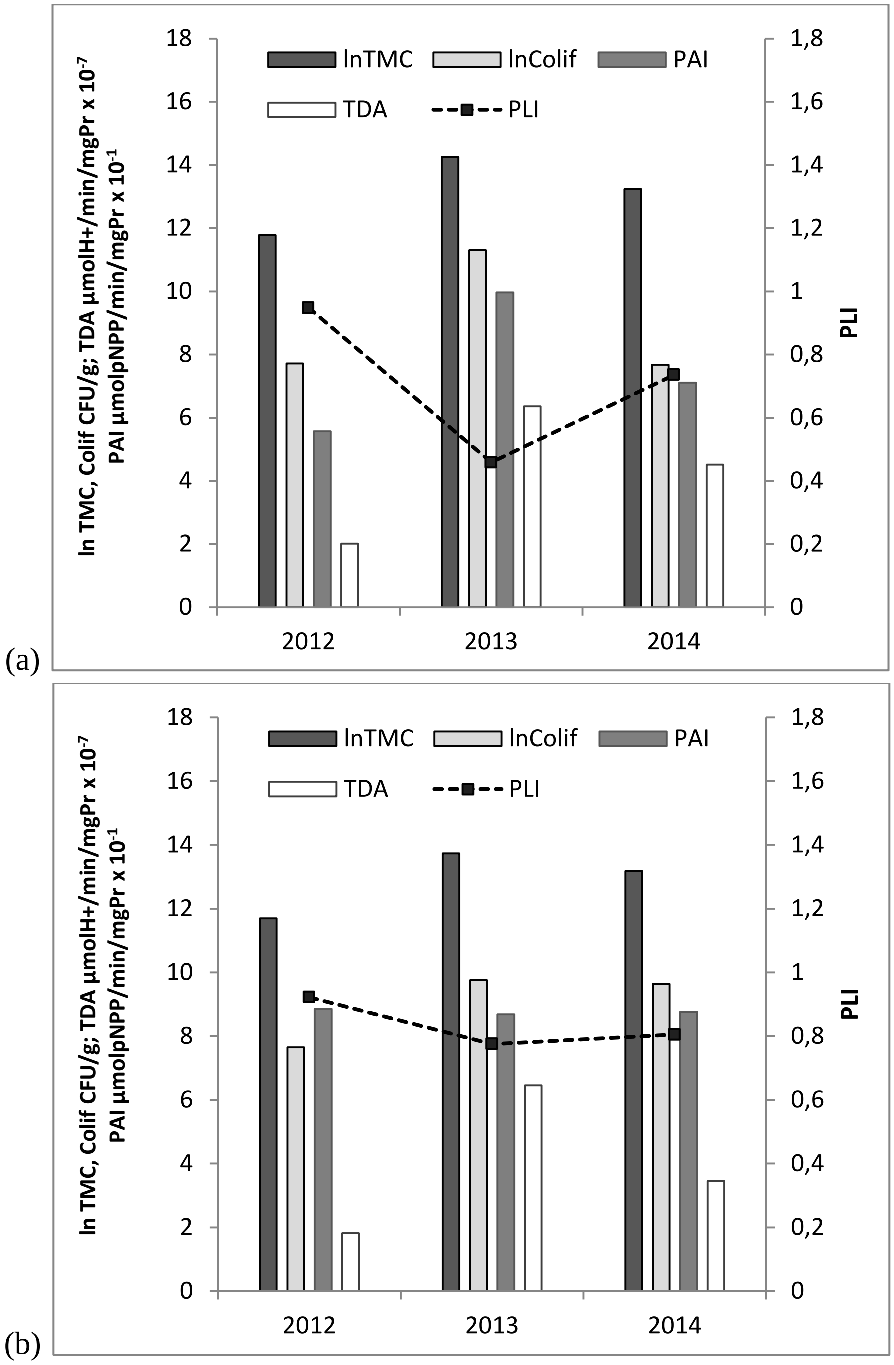

Fig. 3. *Effect of heavy metal pollution (as PLI) on sediment microbial parameters in river sites: (a) Prokopanik; (b) Gabrovnitza*

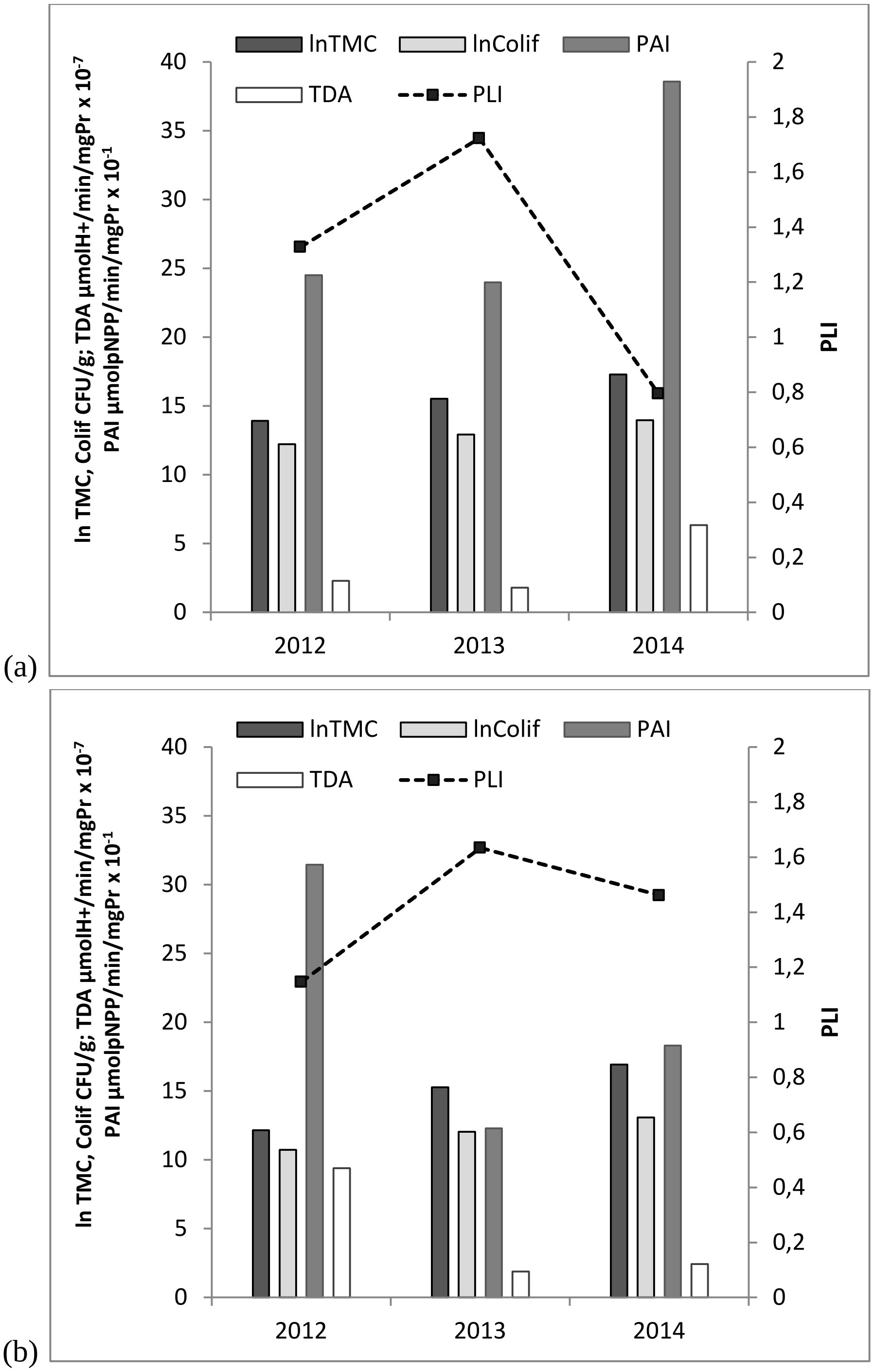

Fig. 4. *Effect of heavy metal pollution (presented as PLI) on sediment microbial parameters in dam sites: (a) Cerovo; (b) Lakatnik*

This strong negative relationship was detected in dam sites too but only for functional parameters (Fig. 4). The total microbial count and coliforms were not affected clearly of high metal content and no correlation was observed with PLI. Probably the permanent higher concentrations of heavy metals in dams' sediments have a role of adaptation factor for bacteria and the sediment microbial community is more resistant on structural level. Their quantitative parameters are less variable and conservative indicators with wide range and long term of reaction. But phosphatase activity index (PAI) and total dehydrogenase activity (TDA) react very sensitively and a decrease of their values was detected at the higher level of pollution in 2013. The two enzyme activities are inhibited by high metal content – the reaction of microbial community is on functional level. The correlation coefficients between PLI and enzyme activities are respectively PAI/PLI= - 0.85 and TDA/PLI= - 0.79.

Conclusion

The construction and operating of SHPs cascade have a negative environmental impact on sediment status by additional accumulation and retention of sediment-related pollutants, especially in dam sites. The assessment of heavy metal pollution in river-dam sequence of SHPs cascade indicates the higher metal concentrations and high Pollution Load Index in dam sediments. The structural and functional parameters of sediment microbial community in river sites of cascade at low level of pollution are indicators with high potential for assessment of heavy metals impact. But in dam sites, the microbial community is more resistant to pollution and structural parameters react conservatively with long reaction time. The enzyme activities are more adaptive and sensitive indicators for different level of environmental impact in this case. The integration of structural and functional response of sediment microbial community is an important tool for the adequate evaluation of the specific pollutants effect. The two selected enzyme indicators have a key biochemical role in the functioning of ecosystem and can provide target information for real heavy metals impact on functional level. Phosphatases and dehydrogenases are an obligatory part of organism enzymatic set, realize the metabolic reactions involved in oxidative energy transfer and are a good indicator of microbial activity. Dehydrogenase enzymes is known to oxidise organic matter by transferring protons and electrons from substrates to acceptors - these processes are part of general respiration pathways and reflect the integral degradation status of soils and sediments (Doi and Ranamukhaarachchi, 2009). The phosphatases catalyzes the hydrolysis of phosphate esters, released phosphate groups bound in complex organic substrates and are involved in the energy metabolism by providing of inorganic phosphates for ATP-synthesis. From this perspective, the enzymatic approach for evaluation of pollutant effects directly assesses the inhibition of key processes in microbial energy metabolism and hence the essential ecosystem functions as an integral productivity and self-purification capacity. The complex enzyme activities – PAI and TDA have a high potential to be used as reliable indicators for precise assessment of hazardous sediment pollution in complicated ecological situations with cumulative impacts.

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Response of Pasture Grasses to Inoculation With Mycorrhizal Fungi and N-Fixing Bacteria

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Abstract

The effect of inoculation with "Mycosym TRI-TON" biofertilizer applied singly or in combination with symbiotic and non-symbiotic N-fixing bacteria on the plant biomass, N and P content of some pasture grasses and soil and roots acid phopsphatase activity was studied in a pot experiment. Active and effective symbiotrophic associations pasture grasses – AMF – N-fixing bacteria, suitable for agricultural practices in the creation of meadows and pastures were selected. The best synergetic interactions were established for the combinations AMF + *Rh. melliloti* 116 for lucerne, AMF + *Rh. trifolii* 325 for red clover and for gramineous grasses – AMF + *Az. brasilense* 107.

Key words: acid phosphatase activity, AM (Arbuscular mycorrhizal) fungi, forage grass, N-fixing bacteria, N and P content, plant biomass

Introduction

Arbuscular mycorrhizal fungi (AMF) form trophic root associations with plants, improving the assimilation and transportation of nutrients from the soil to host plants (Barea, 1991; Grogan and Chapin, 2000; Atkinson et al., 2002; Lopez-Gutierrez et al., 2004). This is due to their capacity to form a lot of branched extracellular structures, which strongly increase the absorbing surface. At the same time the fungal hyphae produce acid and alkaline phosphatases, which dissolve insoluble phosphates, making them available to the plants (Smith and Smith, 1996; Sato et al., 2015). Besides that, the legumes form symbiothrophic associations with symbiotic N-fixing bacteria. In this three-parted association plant-AMF-Rhizobium N and P are the limiting elements. The plant roots and fungal exudates also stimulate the development of free-living N-fixing bacteria. In this trophical associations the microsymbionts mutually support themselves with the main biogenic elements (N, C and P). The positive effect of double and triple inoculation on the quantity and quality of plant biomass of great deal of forage legumes and grasses is proven by many authors (Saini et al, 2004; Miyanchi et al., 2008; Stancheva et al., 2008; Djonova et al., 2011).

Plant species have different capacity to form symbiotrophic associations, which determines the necessity of studies for selection of proper participants in the synergetic interaction between plants, AMF and N-fixing bacteria. The application of plant-microbes associations in the pastures and meadows formation can contribute to an increase of soil fertility and hay production and protect the soil from erosion processes. The utilization of biological methods for improving the plant nutrition ensures receiving of clean plant production on one hand and on the other – protection of groundwater from fertilizers contamination.

The aim of the study was to determine the effect of inoculation with mycorrhizal biofertilizer "Mycosym TRI-TON" applied singly or in combination with N-fixing bacteria on the growth of pasture legumes and gramineous grasses, the N and P content of plant biomass and phosphatase activity.

Materials and Methods

The study was carried out under the conditions of pot experiment with orchard grass (*Dactylus glomerata*), lucerne (*Medicago sativa,* L*.),* red clover (*Trifolium pretense* L*.),* tall fescue (*Festuca arundina* Schreber) and timothy grass (*Phleum pretense* L*.),* grown on Grey forest soils (Orthic Luvisols) (IUSS Working Group WRB, 2006) from the city of Troyan. Pots containing 1 kg of soil were used in the experiment. The agrochemical properties of soil were as follows: humus – 2.07%; NH_4N – 10.0 mg/100 g; NO_3-N – 7.0 mg/100 g; P (P_20_5) – 2.3 mg/100 g; K (K_2O) – 17.8 mg/100 g; pH $_{(KCL)}$ – 5.5. The experimental scheme included inoculation of the plants with mycorrhizal granular "Mycosym TRI-TON" biofertilizer, applied singly or in combination with N-fixing bacteria. The biofertilizer contained mycorrhizal fungus *Glomus intraradices* consisted of more than 50 spores/g and more than 200 IMP (infective mycorrhizal propagules). It was added to the soil (at 2 cm depth under soil surface) before sowing in quantity 0.5 g/pot. The seeds were previously treated with suspension of N-fixing microorganisms (1.10^8 cells/ml) as follows: red clover – *Rhizobium melliloti* 166 or 116; lucerne - *Rhizobium trifolii* 294 or 325 and gramineous grasses – *Azospirillum brasilense* 107 or *Azospirillum lipoferum* 14. The strains tested are from the collection of Soil Microbiology Department of Nikola Poushkarov Institute of Soil Science, Agrotechnologies and Plant Protection. The plants were cultivated up to beginning of flowering. The dry shoot and root biomass were determined at the end of the study.

The N content of the roots and stems was determined by Kjieldahl digestion (Bremner and Mulvaney, 1982). The P content of the same plant parts was analyses by the molybdenum-vanadate method (Olsen and Sommers, 1982). The percentage of mycorrhiza infection of the roots was determined microscopically (Giovanetti and Mosse, 1980). To visualize the AMF colonization, roots were cleared by boiling for 4 min in 10% KON, rinsed three times with tap water, and acidified with 0.1% HCl. Roots were stained boiling in 0.05% Trypan blue for 10 min. Following staining, the roots were rinsed with tap water, mounted on slides, and observed directly in lactic acid. The acid phosphatase (APA, E.C. 3.1.3.2) activity in both rhizosphere soil and roots of control variants and of those, treated with “Mycosym TRI-TON” was measured according to the method of Schneider et al. (2000), based on the original one of Tabatabai and Bremner (1969). Root tissue was homogenized with 0.1 M sodium acetate buffer (pH 5.0). After centrifugation, the supernatant was assayed for the enzyme activity by incubation in 5mM p-nitrophenyl phosphate and 0.1 M sodium acetate buffer (pH 5.0). The reaction was stopped by the addition of 0.2 M NaOH, and absorption was measured at 405 nm. The acid phosphatase activity was determined by colorimetric estimation of the *p*-nitrophenol released by phosphatase activity. Soil sample (1 g) was incubated at 37 C for 1 h with buffered (pH 6.5) sodium *p*-nitrophenyl phosphate solution and toluene. After incubation, the flask was immediately placed on ice and then 1 ml of 2 M $CaCl_2$ and 4 ml of 0.2 M NaOH were added to terminate the reaction and extract the *p*-nitrophenol formed. Absorbance of released *p*-nitrophenol was determined spectrophotometrically at 405 nm. For the blank, *p*-nitrophenyl phosphate was added after the incubation. The acid phosphatase activity was expressed as μmol *p*-nitrophenol per gram dry matter and incubation time 1h (μmol *p*-nitrophenol g^{-1} dry matter h^{-1}).

The statistical processing of the data on plant dry biomass included determination of the least significant differences (LSD) ($P \leq 0.05$) among the treatments. A statistical software package (StatGraphics Plus, version 5.1 for Windows, USA) was used.

Results and Discussion

The tested biopreparation “Mycosym TRI-TON” exerted positive influence on the plant biomass weight in all experimental cultures (Table 1). The different plants demonstrated specificity in their interaction with the microsymbionts. In lucerne, the dual inoculation with “Mycosym TRI-TON” and *Rh. meliloti* 166 showed the highest and significant effect on the dry shoot weight compared with the variant with single mycorrhiza inoculation. The differences among the treated variants were not established in red clover. The effect of the treatments on the dry root biomass compared with the control was significant but the differences among the treatments were not statistically proven. These data are in accordance with the higher percentage of root mycorrhization, obtained for the treated plants. In orchard grass and timothy grass the highest values of dry shoot biomass were established by the variants with dual inoculation with Mycosym and *Azospirillum brasilense* 107. The symbiotrophic association in timothy grass showed significant effect compared with the single inoculation with Mycosym for both dry shoot and root biomass. Similar data for the pointed out treatment were established also for the percentage of root mycorrhization, which was increased about 20% compared with the control and 10% compared with the variants with inoculation. The physiological influence on the tall fescue root biomass was observed in the dual inoculation *Azospirillum lipoferum* 14 + Mycosym.

Table 1. *Shoot and root dry biomass and root mycorrhization*

Treatments	Shoot dry biomass (g/pot)	Root dry biomass(g/pot)	Root mycorrhization(%)
Lucerne			
Control	1.00a	1.23a	38.97
+ Mycosym	1.25b	1.70b	42.70
+Myc.+Rh. Melliloti 116	1.45bc	1.60b	47.08
+ Myc. +Rh. melliloti 166	1.57c	1.60b	42.93
LSD P ≤ 0.05	0.25	0.20	
Red clover			
Control	1.32a	1.00a	41.88
+ Mycosym	2.03b	1.27b	49.41
+ Myc. +Rh.trifolii 294	2.05bc	1.29b	43.00
+ Myc. +Rh. trifolii 325	1.95c	1.16b	47.65
LSD P ≤ 0.05	0.31	0.12	
Orchard grass			
Control	1.15a	1.39a	24.33
+ Mycosym	1.85b	1.72b	36.88
+ Myc. + Az. brasilense 107	1.90b	1.63ab	45.95
Myc. + Az. lipoferum 14	1.43a	1.52ab	40.96
LSD P ≤ 0.05	0.31	0.25	
Timothy grass			
Control	1.30a	0.80a	23.92
+ Mycosym	1.75b	1.24b	37.15
+ Myc. + Az. brasilense 107	2.13c	1.63c	46.14
Myc. + Az. lipoferum 14	1.73b	0.97a	43.82
LSD P ≤ 0.05	0.37	0.18	
Tall fescue			
Control	1.30a	1.07a	25.01
+ Mycosym	1.85b	1.22a	34.96
+ Myc. + Az. brasilense 107	1.95b	1.28a	45.85
Myc. + Az. lipoferum 14	1.78b	1.53b	39.93
LSD P ≤ 0.05	0.25	0.24	
Values in the same column, followed by different letters are significantly different at P ≤ 0.05			

The combined treatment Mycosym + *Azospirillum brasilense* 107 lead to the highest percentage of root mycorrhization but the symbiotrophic association of the two mycrosimbionts influenced only the weight of shoot biomass without that of the roots.

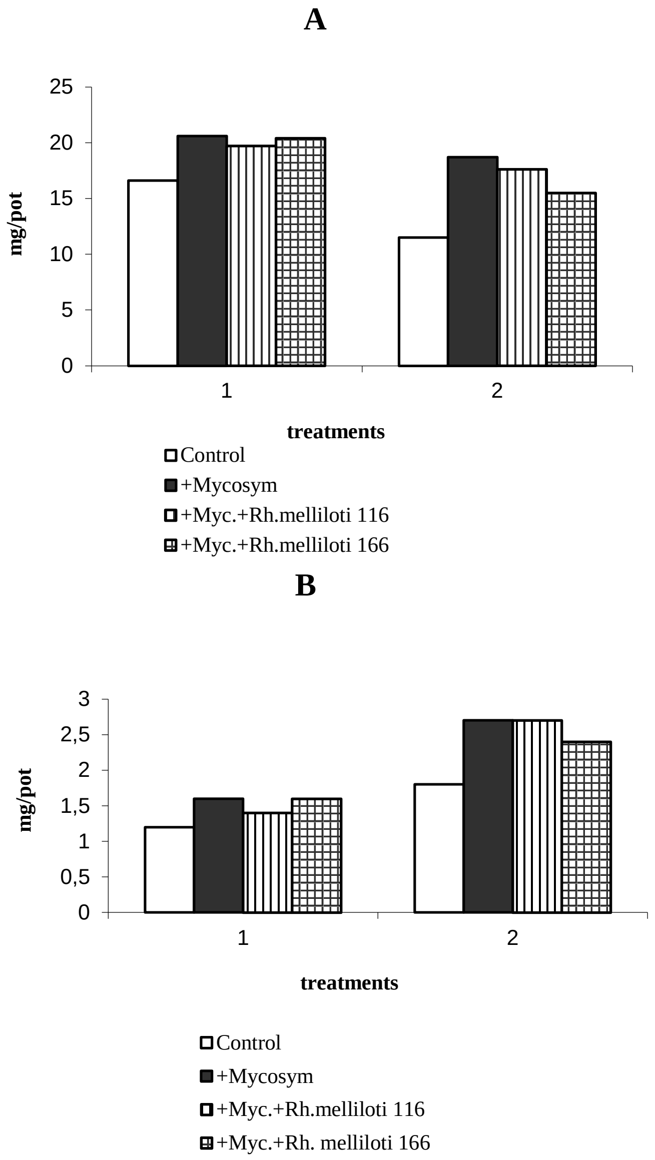

Figure 1. (A) *Nitrogen and (B) phosphorus content of shoots (1) and roots (2) of lucerne*

The data presented in Figures 1-5 show the N and P uptake in plant biomass at the end of the experimental time (beginning of flowering). Specificity of their distribution in shoot and root biomass for different plant species was observed. For both leguminous grasses root and shoot N content was higher in the treated plants compared with the control (Fig.1 and 2). The N content in the shoots was higher than in the roots. This was clearly expressed in red clover, especially in the shoots, where the N content in the variant with dual inoculation AMF + *Rh. trifolii* 325 had the highest value. There were not significant differences in the N content in the variants with single and mixed treatment for lucerne, which indicated that in these variants the mineral nutrition was mainly improved due to the applied treatments. It was established that the AMF-application improved the P nutrition of both plant species tested. The P content of lucerne was higher in the roots than in the shoots while in the red clover the phosphates were exported in the shoots. Again the effect of the dual inoculation AMF + *Rh. trifolii* 325 was the highest concerning the shoot P content.

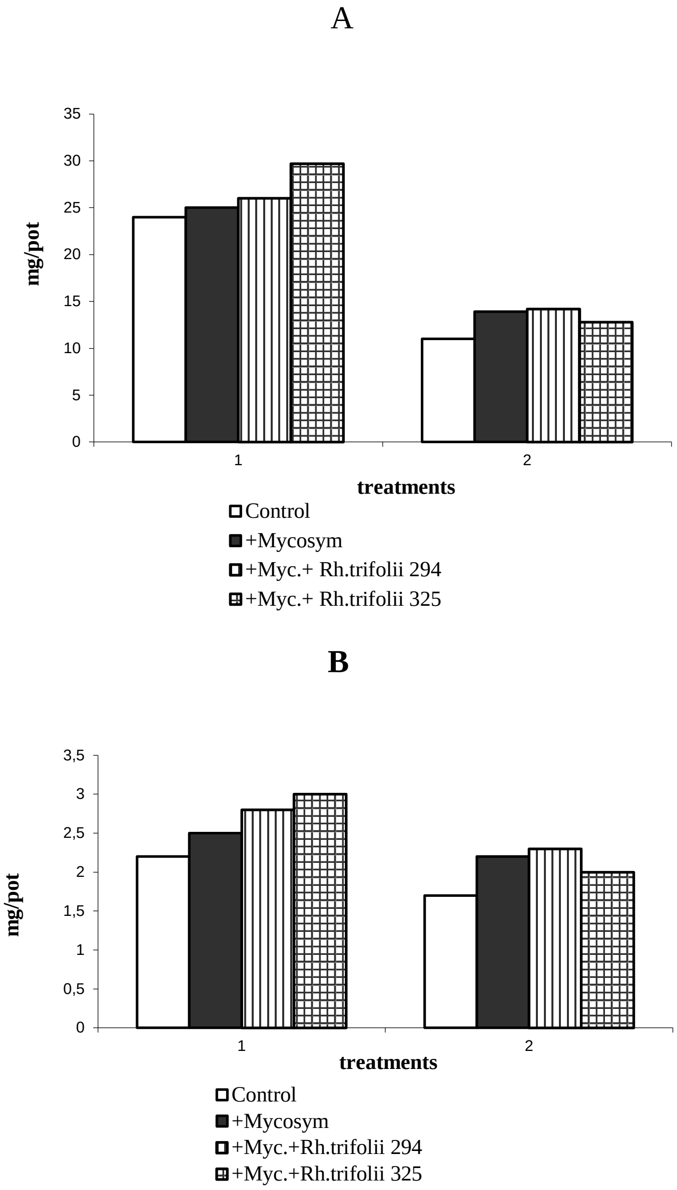

Figure 2. *(A) Nitrogen and (B) phosphorus content of shoots (1) and roots (2) of red clover*

In the gramineous grasses in all treated variants the values of biomass N and P content were higher in comparison with the control and these elements were mostly concentrated in the shoots than in the roots (Figures 3, 4 and 5). The highest values of N and P shoot content were obtained in the variant with dual inoculation, included AMF + *Az. brasilense* 107 for the three pasture grasses.

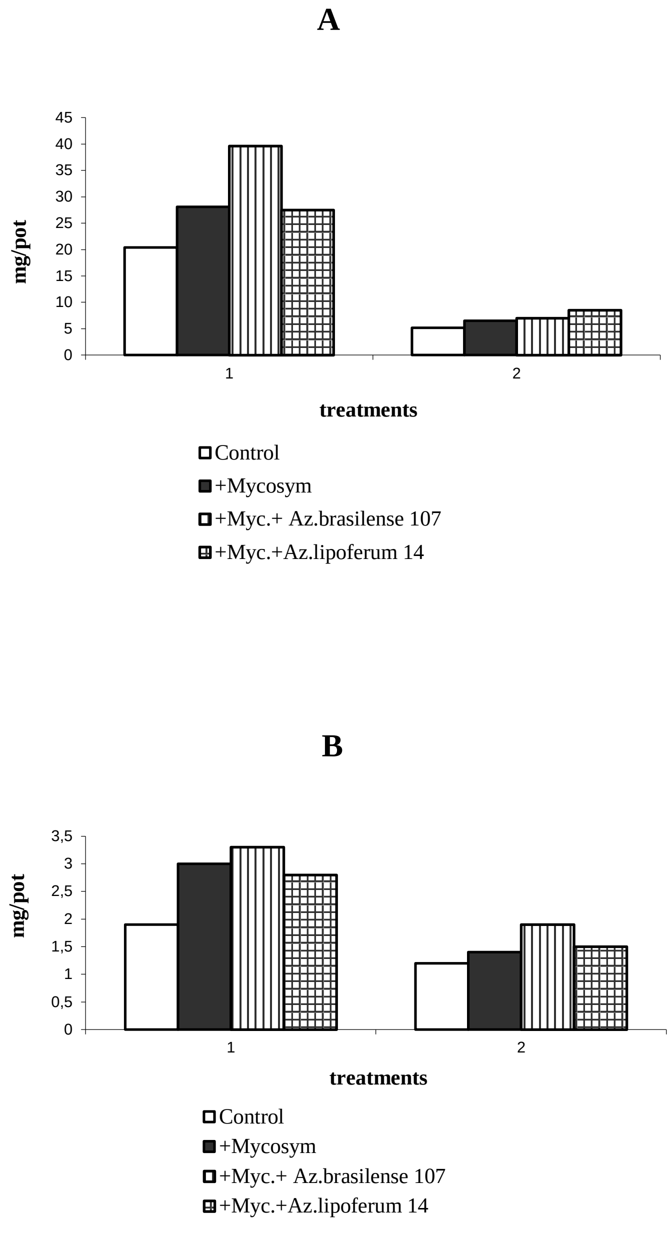

Figure 3. *(A) Nitrogen and (B) phosphorus content of shoots (1) and roots (2) of orchard grass*

The data on soil and roots acid phosphatase activity are presented in Figures 6 and 7. In the gramineous grasses the values of acid phosphatase activity in the roots were considerably lower compared with the leguminous plants. These values were comparatively higher in tall fescue and orchard grass. From both leguminous pastures higher values were obtained in the red clover. These data are in accordance with the higher percentage of the red clover root mycorrhization and considerably higher increase of shoot biomass (53 %) (Table 1). Similar data were obtained in our previous study with the same plant species, grown on slightly eroded Leached Cinnamonic soil (Djonova et al., 2014). Considerable differences in the values of soil acid phosphatase activity among plant species tested were not established but in all treated variants they were higher in comparison with the untreated plants.

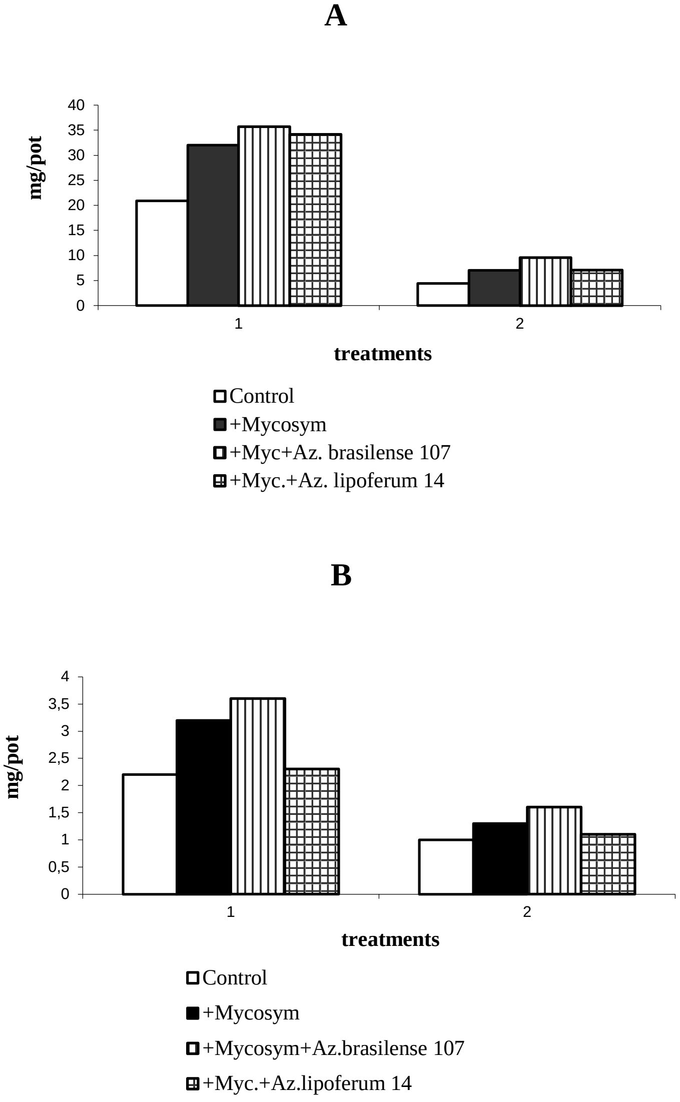

Figure 4. *(A) Nitrogen and (B) phosphorus content of shoots (1) and roots (2) of timothy grass*

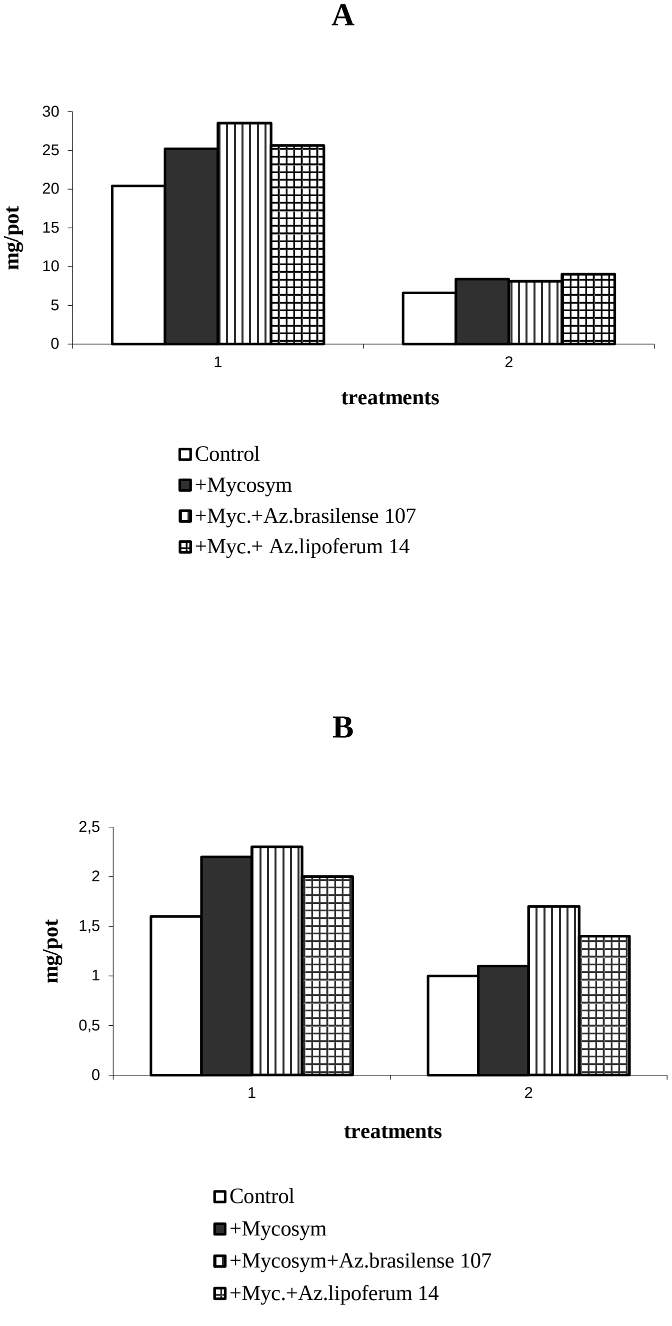

Figure 5. *(A) Nitrogen and (B) phosphorus content of shoots (1) and roots (2) of tall fescue*

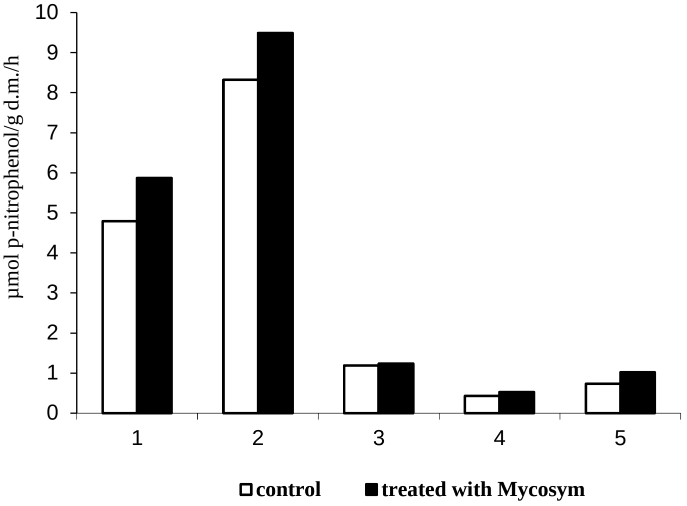

Figure 6. *Acid phosphatase activity in the roots of pasture grasses: 1-lucerne; 2-red clover; 3-orchard grass; 4-timothy grass; 5-tall fescue*

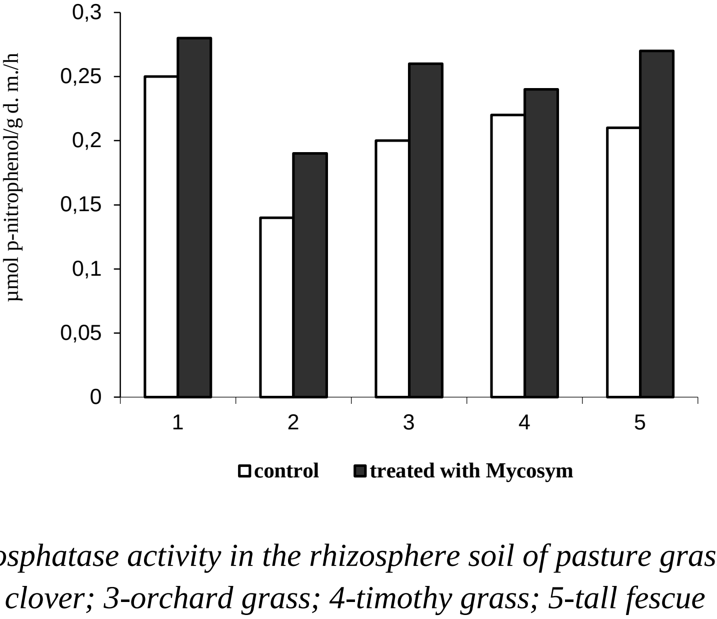

Figure 7. *Acid phosphatase activity in the rhizosphere soil of pasture grasses: 1-lucerne; 2-red clover; 3-orchard grass; 4-timothy grass; 5-tall fescue*

Nitrogen-fixing bacteria (symbiotic, free-living and associative) and AM fungi are plant beneficial microorganisms having the potential to improve biomass production. Their utilization for plant inoculation contributes to soil fertility conservation, reduction of mineral fertilization and ecological safe plant production. They are used as bioinoculants in many countries (Xavier and Germida, 2003; Barea et al., 2004; Perfanova, 2011; Singh et al., 2011; Hungria et al., 2013). The combined inoculation with AM fungi and N-fixing bacteria could have different effect on plant growth depending on the type of microsymbiontic interactions. Mycorrhizal colonization can affect root exudation and hence the composition and function of rhizobacterial community. Some authors reported for competition between AM fungi and Rhizobial bacteria for sites of root penetration (Barea, 1987; Jakobsen et al., 1992). Catford et al. (2003) showed that an established symbiosis in alfalfa could systematically exerted an inhibition on the second one. This inhibition could be explained by the common regulating signals between plant and both AM-fungi and Rhizobial bacteria. It has been established that root flavonoids are the molecular signals involved in the general recognizing process between plant and microorganisms (Juge et al., 2012).

On the other hand there is much information confirming that AM-fungi improve growth and N-fixation in symbiotrophic association plant – N-fixing microorganisms (Valdenegro et al., 2001; Saini et al., 2004; Djonova et al., 2014). This effect is due to the fact that N-fixation depends on steady adequate supply of phosphates to roots. AM fungi are able to take up phosphates from soil solutions with low phosphate concentrations more efficiently than simple roots. They take up, accumulate and transfer large amounts of phosphates to the plant by releasing the nutrients in root cells containing arbuscules. From there phosphates are transferred to cortical root cells, ready to be used by plants. The interactions in symbiotic system AM fungi – N-fixing microorganisms – plant are complex and that is why empirical studies should be carried out for each concrete symbiotrophic association.

In our study an increase of root and shoot biomass and their N and P content was established, which demonstrated synergetic interaction between tested AM fungi and Rhizobial strains. Obviously, plants recognized inoculants as beneficial partners. The effect of mixed application of “Mycosym TRY-TON” showed specificity for the tested plant species and depended on the type of interaction among microsymbionts. The best synergetic interations were established for alfalfa, treated with AMF + *Rh. melliloti* 116 and for gramineous grasses – AMF + *Az. brasilense* 107.

Many authors reported that AM-fungi increase the acid phosphatase activity in roots and rhizosphere zone (Bolan et al., 1987; Tarafdar and Marschner, 1994; Sato et al., 2015). The data presented in Figures 6 and 7 confirm the stimulative effect of mycorrhizal inoculation on acid phosphatase activity. The higher values of root acid phosphatase activity by the pasture legumes corresponded to their higher mycorrhization and better supply of the plants with biologically fixed nitrogen. The level of soil phosphatase activity is influenced by the root exudates, which from the other hand, also influence on the activity of the free-living soil phosphate-decomposing bacteria. There were no considerable differences of the values of soil acid phosphatase activity among plant species tested, which could be due to the vegetation stage of the plants at the end of the experimental time (Figure 7). Probably, in later stages they may occur depending on the specificity of the composition and quantity of the exudates of the tested plants.

The obtained results outline active and effective symbiotrophic associations pasture grasses – AMF – N-fixing bacteria, suitable for agricultural practices in creating of meadows and pastures. These areas are situated mostly in mountains on eroded soils in drink water supplying zones. The application of such biological methods ensures not only higher quality and quantity of plant production but also protects the soil from erosion and contamination.

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New Holistic Approach for Tree Eustress Assessment and Influenced Climatic Types Classification (Example with Mountain Forest Trees in Bulgaria)

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Abstract

The paper deals with meta-analysis of 259 dendrochronological series of mountain trees – Bosnian pine, Norway spruce and beech from 14 locations in Bulgaria for eustress investigation. The chronologies were analyzed by the original SPPAM 2.0 application. The tree ring width sequences were approximated with polynomials with determination coefficients, $\boldsymbol{R^2} \geq 0.45$. The growth index (***It*** – the ratio between measured and approximated value of tree ring width) was computed. Years with ***It*** less than the threshold values were considered as eustress ones. The four grade scales for assessment of frequency, duration and depth of eustress was proposed. For the studied tree species and locations, the obtained eustress periods are as follows, respectively for the Bosnian pine, Norway spruce and beech: 42 (K=9.1), 30 (K=3.4) and 37 (K=3) on average. The obtained eustress characteristics are respectively: Average Depth, $\boldsymbol{A_{av}}$ = 0.16, 0.26 and 0.28; Average Duration, $\boldsymbol{D_{av}}$ = 2, 2.5 and 2.3 years; and Average Frequency, $\boldsymbol{F_{av}}$ = 10, 33 and 34 years over 100 years. The risk for The stands of two studied locations of spruce and three locations of beech were established to be in some risk. They require further monitoring. The predominance of cold-dry and hot-wet years in total number of adverse climatic years (with eustress) for the Bosnian pine, as well as hot-dry and cold-wet years for spruce and beech stands was established. The influence of climatic types of years on the eustress appearance for tree years periods have also been investigated. Proposed holistic approach for meta-analyses of series (eustress identification in radial growth of stems) is convenient for the fast monitoring of forest communities state and for recognizing the stands under risk. The capability of approach allows expression of reactive functional types of trees (PFTs) as groups of trees with the similar behavior to the influence of climatic types of years, and with similar characteristics of eustress periods. These PFTs can support the development of different local, regional and global models. The originality of approach applied includes: meta-analysis, new software, characteristics, indixes and coefficients.

Key words: meta-analysis, eustress, climatic type of year, classification, dendrochronology, SP-PAM application, *Pinus heldreichii* Christ., *Picea abies* (L.) Karst., *Fagus sylvatica* L.

Introduction

Mountain ecosystems are among the most sensitive to environmental changes (Lloyd 1997, Körner 1998, Moiseev 2002, Camarrero et al. 2003, Esper et al. 2004). This is one of the reasons, why many studies on climate change have been focused on treeline forests (Panayotov et. al. 2008). Among the frequently used research method for the forest-climatic impact investigations is dendrochronology (Fritts 1976, Schweinguber 1996, Briffa et al. 2001). The studies are based on the tree rings, which formation is strongly dependent on environmental factors and especially on the temperature and precipitation (Fritts 1976). Once formed, tree rings do not change with time makes tree-ring chronologies a “natural archive”, containing information on past climate and historical development of individual sites. In the last decades numerous proxy climate reconstructions based on tree line sites from high elevation and northern environments, have been created for various parts of the world (e.g., Briffa 2000, Esper et al. 2002, Cook et al. 2004, D’Arrigo et al. 2006). Limited knowledge is available about the growth of Norway spruce - saplings related to spring climate, especially snow duration, in high mountain forests**.** After conducting tests, the impact of above-mentioned factors was proven as very important for the growth of Norway spruce saplings (Cunningham et. al. 2006). Other studies of *Abies alba* Mill. and *Picea abies* (L.) Karst. have found out that the precipitation in the first period of the growing season, i.e. early spring, is a major factor influencing the annual growth of native *A. alba* trees, although they proved to be sensitive to drought. *P. abies* showed a higher tolerance to summer drought stress. According to Henne et. al. (2011) temperature alone cannot account for important changes in tree species abundance. For example, population expansion by Norway spruce (*Picea abies*), a dominant higher mountain species, link with lagged suitable temperatures by about 3000 years in eastern and by 6000 years in western Switzerland. Trackbacks study of *P. abies* for the link between individual factors, incl. growth, and mortality in space and time are resulted in development of more accurate mortality models and increasing the reliability of forest dynamics predictions (Bigler et al. 2004). The influence of waterlogging on growth of common beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) has been studied intensively on seedlings under experimental conditions. The results indicate that under the projected future climatic scenarios, beech may suffer from increasing drought stress even on hydromorphic soils. Oak might be able to maintain a sufficient hydraulic status during summer droughts by reaching water in deeper soil strata with its root system. Wet phases with waterlogged soil conditions during spring or summer appear to have only a little direct influence on radial growth of both species (Scharnweber et. al. 2013).

Recently, researches into new holistic approach to assess the “behavior” of tree species and their communities under the climatic influence (temperature-precipitation patterns) by Lyubenova (2012), Lyubenova et al. (2014ab) and others were published. In this approach the eustress periods in radial growth of trees as the statistically proven reduction in the tree rings width, reflecting the overall result of the interactions tree species - community – environment have been analyzed. The multi-annual survey of eustress periods and their assessment by frequency, duration and depth (using developed scales) allows for categorization of communities, risk assessment and the sustainable existence of forest areas prediction. The relationships between eustress periods and temperature-precipitation patterns by defining the climatic norms (CN) in temperature and precipitation and climatic types of years (CTYs) have also been tested. CNs are calculated as average values of annual temperatures (CNs for T) and annual sum of precipitations (CNs for P) for 30 – years' periods. CTYs are defined according to the annual T deviations from CNs for T and annual P deviations from CNs for P. The defined in this way CTYs are nine: hot and dry (HD), hot and wet (HW), hot with precipitations in the norm (HN), cold and dry (CD), cold and wet (CW), cold with precipitations in the norm (CN), with temperatures in the norm and wet (NW), with temperatures in the norm and dry (ND), with temperatures and precipitations in the norms (NN). These studies have the origin of meta-analysis of dendrochronology and climatic data from various international bases and own research. For this purpose, the SP-PAM software has been developed, which includes classic dendrochronology analyzes and novel ones. The application is evolved in several versions (Lyubenova et al. 2015). So far, the results of approach applied to four conifer and 5 deciduous species from 102 locations in Europe (total 1674 dendrochronological sequences, incl. 171 from Bulgaria) have been published. These investigations should help the global forest monitoring, modelling and prediction, as well as should help to define criteria for sustainability - effective forest management, forest services usage and conservation. The main goal of present research is to apply the holistic approach for eustress periods identification and valuation for 3 mountain tree species, as well as to study their relationships with climatic types of years for included locations, and comparative analysis of results for these species in the light of upcoming climatic changes.

Materials and Methods

Analyses were conducted for 259 dendrochronological series of 3 mountain tree species in 14 Bulgarian locations – 3 in Pirin Mount. for *Pinus heldreichii* Christ., 4 in the Balkans for *Picea abies* (L.) Karst. and 7 in the Rhodope Mount., Western Balkans, Fore Balkan and Osogovo Mount. for *Fagus sylvatica* L. The average annual temperature ($T_{av.}$) of the selected locations varies from 7.1 to 11.8^0 C; average annual precipitations (P_{avg}) are from 500 to 653 mm – Table 1. The parameters of Bosnian pine chronologies, 74 in number, are: 48 – 655 years age, encompassing the period from 1721 to 2008; DBH (diameter at breast height) is about 26 to 47 cm and EPS (expressed population signal) is from 79 to 85%. The parameters of Norway spruce chronologies, 76 in number, are: 16 – 118 years age, encompassing the period from 1886 to 2011; DBH is about 31 to 38 cm and EPS is from 83 to 96%. The parameters of beech chronologies, 89 in number, are: 42 – 151 years age, encompassing the period from 1853 to 2011; DBH is about 30 to 55 cm and EPS is from 53 to 89%. Despite the studied chronologies do not match by age and do not cover the same periods (matched only for the years 1853 to 2008), monitoring the nature of eustress allows for analysis of the behavior of species under the influence of unfavorable factors for hundreds years and assessment of respective tree ecosystems. It is almost impossible to examine the chronologies with similar parameters even for one species in different locations, unless the plantations will be created and wait 100-200 years for their development.

Table 1. *Object characteristics*

Sampling area	Long.	Lat.	Alt., m	Tav, 0C	Pav, mm	Cl. Period, y		Age$_{min}$, y	Age$_{max}$., y	DBH, cm	Period, y		N	EPS,%
***Pinus heldreichii* Christ.**														
1.Vihren, Pirin Mount.	23.30	41.42	2150	8.8	591.6	1901	2008	48	260	25.7	1721	2008	42	82.95
2.Vihren, Pirin Mount.	23.23	41.46	1920	8.8	602.6	1901	1981	87	261	45.2	1721	1981	22	85.12
3. Pirin Mount.	23.42	41.82	1862	7.1	652.7	1901	2002	103	655	47.3	1348	2002	10	78.65
***Picea abies* (L.) Karst.**														
1.Chuprene W. Balkan	22.37	43.26	1600	10.8	558.1	1901	2002	16	116	37.9	1886	2002	40	93.26
2.Yundola, Rila Mnt.	23.50	42.02	1400	8.8	590.3	1901	2009	31	118	31.3	1894	2011	27	95.70
3.Sipenski prohod, Kazanlak t.	23.00	42.44	1300	9.6	626.4	1922	1981	40	60	32.7	1922	1981	9	83.45
4.Groba, W.Balkans	22.59	43.17	1078	11.7	508.2	1937	2009	63	76	64.7	1937	2012	4	91.14
***Fagus sylvatica* L.**														
1.Chuprene	23.38	43.62	1596	11.5	519.6	1901	2003	80	151	34.6	1853	2003	23	75.43
2.Petrohan, W.Balkans	23.12	43.13	1200	11.8	499.7	1901	2009	42	108	28.4	1900	2010	14	75.97
3.Kyustendil t.	22.38	42.15	890	8.9	597.3	1920	2009	49	92	29.7	1920	2011	13	65.05
4. Brekovitsa t.	23.22	43.22	820	11.8	504.8	1916	2009	77	95	49.1	1916	2010	7	89.40
5. Velingrad t.	23.59	42.01	812	8.6	614.4	1926	2009	54	86	40.9	1926	2011	9	56.94
6.W.Balkans	23.22	43.21	800	11.8	499.8	1901	2008	45	108	33.4	1893	2008	14	71.88
7. Sevlievo t.	25.06	43.02	752	11.6	528.4	1917	2009	39	95	55.1	1917	2011	9	53.19

SPPAM, version 2.0 is applied for the data analysis; its functionality encompasses classical analyses in dendrochronology (Cook 1990, Schweingruber 1996, Fritts 2001, Mirchev et al. 2000) and new developments (Lyubenova et al. 2014). Sequences with $R^2 > 0.45$ and locations with EPS >80% are included in the analysis. The studies on the expression of eustress and its assessment are conducted by analysis of the average growth index sequences (It = MW/AW, where MW is measured tree rings width and AW – computed width, only for the sequences with trusted approximations). The index allows removing the strong influence of age on the radial growth, because of usually different ages of the studied chronologies and the detection of climatic impact. As an eustress year (SY), the year for which more than half of the available sequences (from one location) have It $<1 - \delta_i$ (where δ_i is the confidence interval for a level of significance, $\alpha = 0.05$) was accepted. The eustress is represented of one or more consecutive eustress years and is characterized by: duration (D), i.e. the number of consecutive eustress years which comprises it; frequency (F) – the number of eustress years for 100 years and depth (A) described reduction in growth -

$A = \frac{1}{s}\sum_{i=1}^{s}(1 - It_i)$, where It_i are the growth index sequences for which the eustress has been found. These characteristics were assessed by the creation for each tree species 5-graded scales – Table 2. The additional dendrochronology data for the scales evaluation was used (International Tree Ring Data Base).

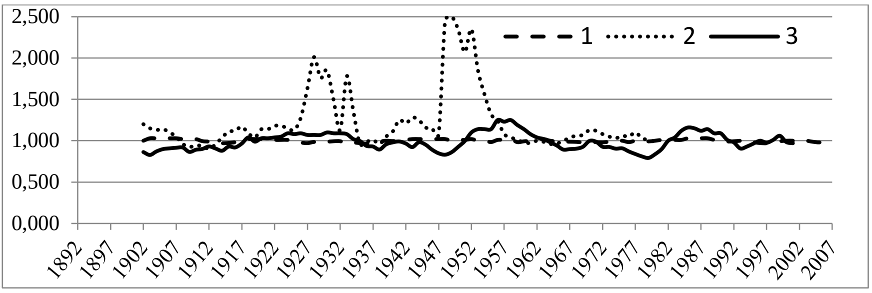
Table 2. *Five-graded scales for assessment of eustress features*

F				D		A		
Group		Value	Group		Value	Group		Value
Pinus heldreichii Christ.								
1	Very rarely	≤1.12	1	Very short	≤1.12	1	Very small depth	≤0.08
2	Rarely	>1.12 ≤4.23	2	Short	>1.12 ≤1.62	2	Small depth	>0.08 ≤0.14
3	Normal	>4.23 ≤10.44	3	Normal	>1.62 ≤2.61	3	Normal depth	>0.14 ≤0.26
4	Offen	>10.44 ≤13.55	4	Long	>2.61 ≤3.11	4	Deep	>0.26 ≤0.32
5	Very Offen	>13.55	5	Very Long	>3.11	5	Very Deep	>0.32
***Picea abies* (L.) Karst.**								
1	Very rarely	≤11.78	1	Very short	≤1.91	1	Very small depth	≤0.19
2	Rarely	>11.78 ≤17.86	2	Short	>1.91 ≤2.15	2	Small depth	>0.19 ≤0.22
3	Normal	>17.86 ≤30.04	3	Normal	>2.15 ≤2.63	3	Normal depth	>0.22 ≤0.26
4	Offen	>30.04 ≤36.12	4	Long	>2.63 ≤2.87	4	Deep	>0.27 ≤0.28
5	Very Offen	>36.12	5	Very Long	>2.87	5	Very Deep	>0.28
***Fagus sylvatica* L.**								
1	Very rarely	≤15.85	1	Very short	≤1.60	1	Very small depth	≤0.25
2	Rarely	>15.85 ≤21.3	2	Short	>1.60 ≤1.88	2	Small depth	>0.25 ≤0.28
3	Normal	>21.3 ≤32.18	3	Normal	>1.88 ≤2.44	3	Normal depth	>0.28 ≤0.33
4	Offen	>32.18 ≤=37.62	4	Long	>2.44 ≤2.71	4	Deep	>0.33 ≤0.36
5	Very Offen	>37.62	5	Very Long	>2.71	5	Very Deep	>0.36

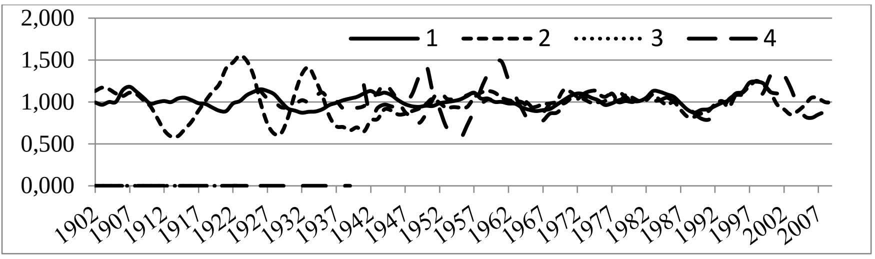
The calculated coefficients are as follows: cardinality (Card) – the number of indexed series of the investigated species with the same stress years; “Ct”- coefficient - the ratio between cardinality (Card) and the total number of analysed sequences from one location (Total n); coverage (Cov) = 100*Card/N, where N is the number of indexed series with measurements for the same periods; “K”- coefficient - the ratio between the number of years for which the analysis was performed (Period) and the number of stress years (SY) by Lyubenova et al. (2014). The climatic type of every year from periods of studied series (CTY) was also calculated using the deviations of average annual temperatures and precipitations from the average values for the 30-years periods and their confidence intervals. The adverse year (AY), or eustress year (SY), was considered as CTY in which the eustress is established. Source of data on temperatures and precipitations was taken from the CRU – TS Climatic database and covers the period 1901-2009.

Results and Discussion

The mean growth index (It) of model chronologies is as follows: for studied locations of Bosnian pine - 1.126 ± 0.166 and ranged from 0.998 ± 0.030 (3 location) to 1.382 ± 0.461 (2); for studied locations of Norway spruce – 1.017 ± 0.082 and ranged from 1.009 ± 0.080 (2) to 1.025 ± 0.095 (4) and for studied locations of beech – 1.041 ± 0.107, ranged from 0.993 ± 0.0059 (6) to 1.203 ± 0.229 (5). The variation of It_{av} by locations is presented in Fig. 1. The synchronicity in the changes of values is weak in general. The directions of change for some locations and periods are different, which is probably due to the local environmental features, the influence of other, non - climatic factors, and different age ranks.



A.



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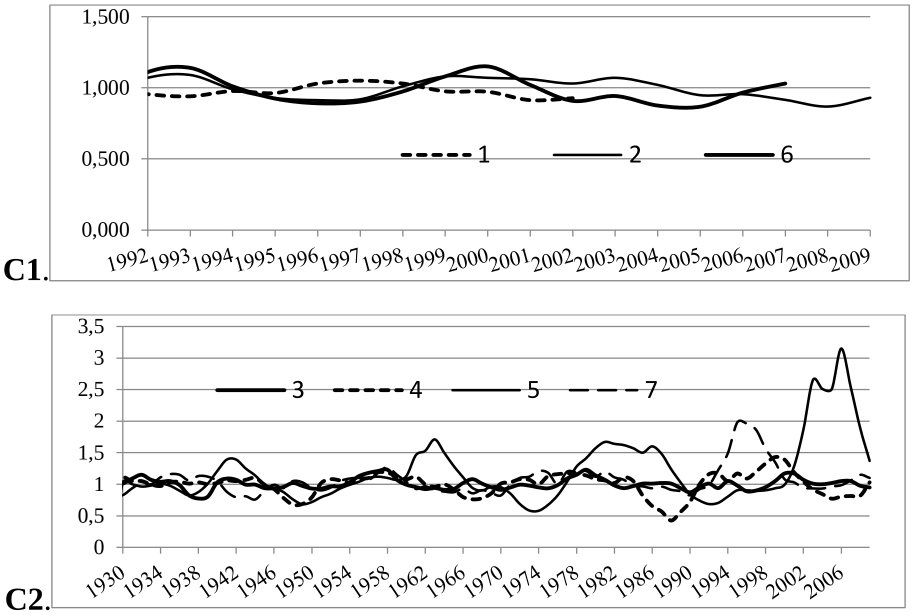

Fig. 1. *Growth index variation by locations and years for: A. Bosnian pine; B. Norway spruce and C. Beech*

As a result of statistical analysis of chronologies the eustress periods were detected for studied species and locations, as follows: for the Bosnian pine - average 42 in number (from 28 to 57), for the Norway spruce – average 30 (from 20 to 46) and for the beech – average 37 (from 20 to 49) – Table 3. Due to the different number of chronologies analyzed and different duration of investigated periods, the results cannot be compared by locations and species. So, the coefficients calculated allow comparative analysis based on a non-homogeneous data. The average Cov is similar for three species series - 0.7 and is ranged from 0.6 to 0.8 by locations. In other words, the homogeneity of the analyzed series by locations is similar - the number of total periods of stress, relative to identical measured periods varies to a small extent. The average Ct values are between 0.5 for spruce and 0.4 for pine to 0.2 for beech. The species and locations with lowest Ct - values are more likely to have a more pronounced influence of stress factors than other studied locations. The average K - coefficient is 9.1 and 3.4 to 3.0, respectively for pine, spruce and beech. The species and locations with maximum K-values are more likely to have more eustress periods in comparison to the others.

The obtained values for the three eustress characteristics are presented in Table. 4. The depth varied in the ranges, respectively for the pine, spruce and beech: 0.058 - 0.212, 0.229 - 0.304 and 0.235 - 0.365. The absolute maximums in A-values was in 1929 (A = 0.379) for the pine, 1951 (A = 0.719) for the spruce and 1990 (A = 0.713) for the beech. The years, in which the maximum values in depth of eustress were established for investigated species, were published as adverse years for other tree species and their communities (Lyubenova 2012). The average values of eustress duration (D) vary from 1 to 3 years. The absolute maximums in D-values for pine and beech were in 1905-1914 and 1972-1981 (10 years) and 1959-1970 (12 years), while for the spruce, the absolute maximums were in previous centuries.

Table 3. *Characteristics of investigated series*

Species / SA	**Period, y**	**It (av)**	**μ (av)**	**SY, n**	**Card**	**Cov**	**Ct = Card/N**	**K = P/SY**
***Pinus heldreichii* Christ.**	**392**	**1.126**	**0.166**	**42**	**7.2**	**0.7**	**0.4**	**9.1**
1. Vihren, Pirin Mount.	260	0.999	0.008	40	6.6	0.7	0.2	6.5
2. Vihren, Pirin Mount.	261	1.382	0.461	28	9.6	0.6	0.4	9.3
3. Pirin Mount.	655	0.998	0.030	57	5.5	0.7	0.5	11.5
***Picea abies* (L.) Karst.**	**93**	**1.017**	**0.082**	**30**	**8.1**	**0.7**	**0.5**	**3.4**
1. Chuprene v., W. Balkan	116	1.021	0.077	46	11.2	0.6	0.3	2.5
2. Yundola, Rila Mnt.	118	1.009	0.080	20	13.6	0.7	0.5	5.9
3. Sipenski prohod, Kazanlak t.	60	1.012	0.076	25	5.2	0.7	0.6	2.4
4. Groba, W.Balkans	76	1.025	0.095	27	2.3	0.8	0.5	2.8
***Fagus sylvatica* L.**	**105**	**1.041**	**0.107**	**37**	**3.1**	**0.7**	**0.2**	**3.0**
1. Chuprene v.	151	0.999	0.062	48	7.1	0.6	0.3	3.1
2. Petrohan, W.Balkans	108	1.000	0.062	49	3.1	0.7	0.2	2.2
3. Kyustendil t.	92	1.005	0.078	23	3.2	0.7	0.2	4.0
4. Brekovitsa t.	95	1.018	0.104	35	2.2	0.8	0.3	2.7
5. Velingrad t.	86	1.203	0.229	20	1.1	0.6	0.1	4.3
6. W.Balkans	108	0.993	0.059	40	3.4	0.7	0.2	2.7
7. Sevlievo t.	95	1.068	0.154	43	1.4	0.7	0.2	2.2

The established absolute maximus for A and D not matched by years, but they were in years identified as eustress years for the investigated species. These years have been proved as adverse years for other tree species and their communities (Lyubenova 2012). The average frequency of eustress seems to be higher in beech (31 to 45 years in a hundred years), then in Norway spruce (16-41) than it in Bosnian pine (8-13 years). The species in surveyed locations exhibit various features on the different characteristics studied. The largest number of SP for equated time was for the B. pine, but they are with the lowest average and maximum depth in comparison to other two species. According to D_{av} – values, the species are slightly different, but the greatest value of absolute maximum is for the beech trees, where and the frequency of SP for 100 years is the greatest.

Table 4. *Characteristics of eustress and functional type (PFT)*

Species / SA	**A_{av}**	**A_{max}**	**$A_{max, y}$**	**D_{av}**	**D_{max}**	**$D_{max, y}$**	**F_{av}, 100 y**	**PFT**
***Pinus heldreichii* Christ.**								
1. Vihren, Pirin Mount.	0.058	0.100	1933	2	5	1911-1915	13	F4D3A1
2. Vihren, Pirin Mount.	0.212	0.379	1929	2	4	1952-1955 1962-1965	10	F3D3A3
3. Pirin Mount.	0.198	0.367	1978	3	10	1905-1914 1972 -1981	8	F3D5A3
***Picea abies* (L) Karst.**								
1. Chuprene, W. Balkan	0.229	0.409	1893	3	7	1890-1896	39	F5D4A3
2. Yundola - Rila Mnt.	0.258	0.335	2001	2	5	1952-1956	16	F2D1A4
3. Sipenski prohod, Kazanlak t.	0.246	0.469	1947	2	5	1936-1940 1945-1949	41	F5D1A3
4. Groba, W.Balkan	0.304	0.719	1951	3	6	1951-1956 1962-1967	35	F4D5A5
Fagus sylvatica L.								
1 .Chuprene v.	0.262	0.376	1910	2	5	1962-1966	31	F3D3A2
2. Petrohan, W.Balkans	0.235	0.524	1917	3	9	1913-1921	44	F5D4A1
3. Kyustendil t.	0.293	0.546	1993	1	2	1942 -1943 1950-1951 1973-1974 1989 -1990 1995-1996 2002-2003 2008-2009	25	F3D1A3
4. Brekovitsa t.	0.365	0.708	1988	2	6	1985-1990	36	F4D3A5
5. Velingrad t.	0.241	0.602	1972	3	6	1988-1993	23	F3D5A1
6. W.Balkans	0.265	0.524	1917	2	5	1915-1919	34	F4D3A2
7. Sevlievo t.	0.284	0.713	1990	3	12	1959-1970	45	F5D5A3

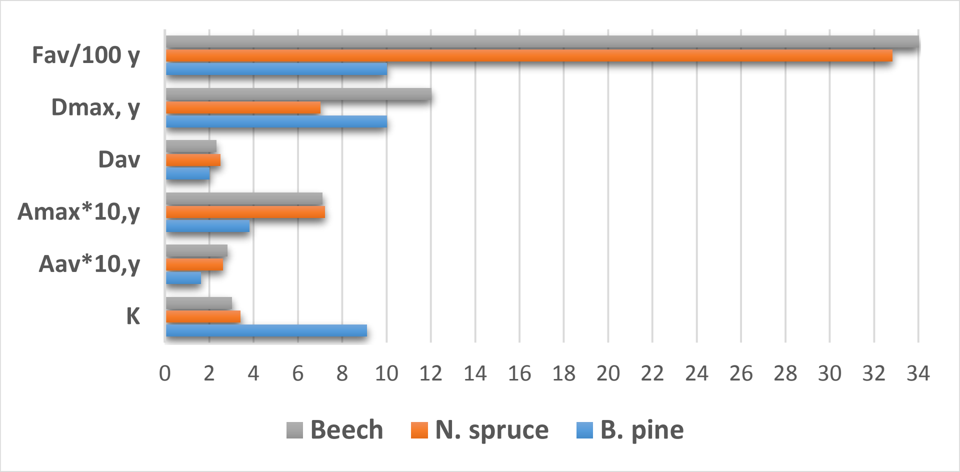

Fig. 2. *Comperative analysis of species by studied ckaracteristics*

The received functional types for the most of locations showed no risk for the forests of studied species. The risk exists if at least two characteristics are valuated with 4 th or 3 th balls. For the 1st and 4th locations of spruce, the obtained functional types are respectively: F5D4A3 (SP appeared very often and with long duration) and F4D5A5 (SP appeared often, with very long duration and are very deep). For the 2 th, 4th and 7th locations of beec, the obtained functional types are respectively: F5D4A1 (SP appeared very often, with long duration and with very small depth), F4D3A5 (SP appeared often, with very long duration and are very deep) and F5D5A3 (SP appeared very often, with very long duration). The forests in these locations are in need of state monitoring, especially the spruce community in location 4.

Table 5. *Correlation coefficients between growth index (It) and used climatic parameters*

Species/Parameter	**It, T_{av}**	**It, P_{av}**	**It, dT**	**It, dP**
Pinus heldreichii Christ.				
Av	0.024	-0.084	0.032	-0.101
Picea abies (L) Karst.				
Av	0.022	-0.065	-0.009	-0.054
Fagus sylvatica L.				
Av	0.003	-0.013	-0.041	-0.010

According to the correlation analysis, the estimated average correlation coefficients between the Itsequences and climatic parameters (Tab. 5), it is observed very weak correlation, ie the influence of climate on radial growth can not be analyzed, however, it was found the climatic dependence by physiological studies. So as to receive an objective picture about the climatic influence on the radial growth and obtained eustress, the climatic types of years was identified for studied periods and locations and their percent participation was calculated. The participation of different CTYs very much vary by locations, because of variation of their geographical and climatic parameters. The significance of CTYs for eustress appearance is presented in Fig. 3. For the Bosnian pine studied locations, cold - dry (CD) and hot – wet (HW) CTYs have the most eustress provoked influence, followed by hot – dry (HD) and cold – wet (CW) years. For the Norway spruce and beech studied locations, the opposite can seen - a paramount importance have HD and CW climatic types, followed by CD and HW types.

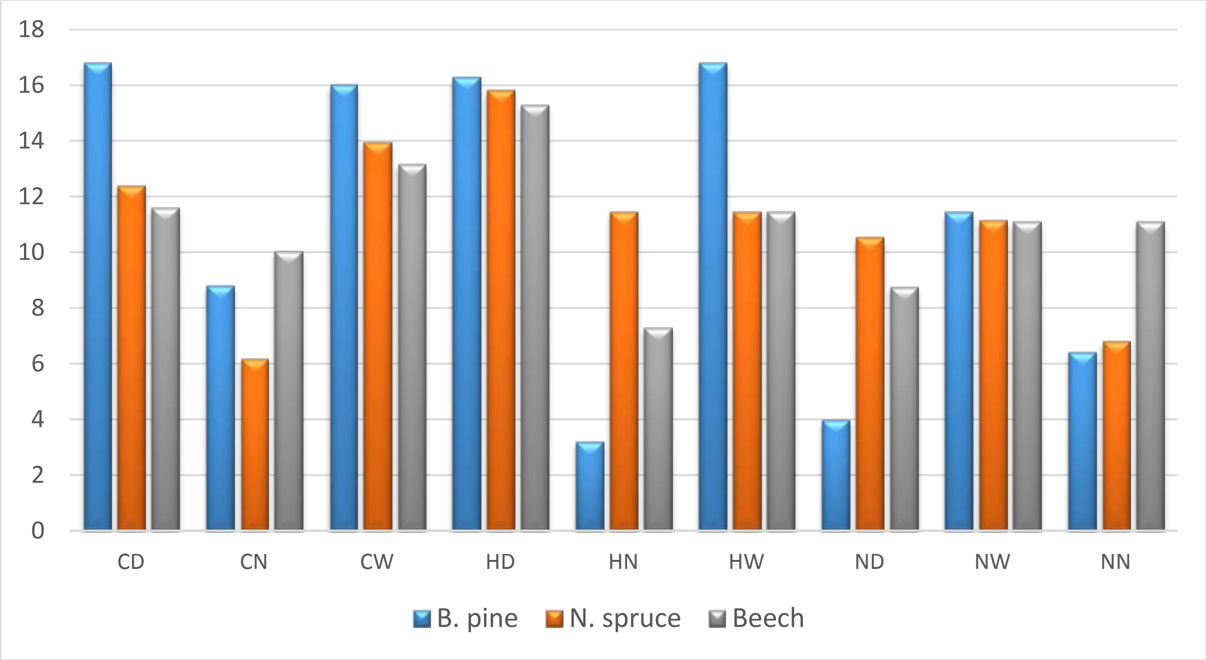

Fig. 3. *Eustress significance of different climatic types (%)*

As eustress climatic types (Fig. 3) the years with temperatures and precipitations in the climatic norm were involved, respectively 6.4%, 6.8% and 11.1% for the Bosnian pine, Norway spruce and Beech. That in many cases is due to the response inertia of tree species - the existence of eustress in the one or two previous years. Where this is not the case, eustress probably is caused by the other environmental factors, rather than climate change. So, the stress influence of climatic type depends on the temperature and precipitation in previous years - the accumulation of influence, also on the degree of deficiency of heat and humidity, and can be the result from the impact of other factors. Thus, the sequences of climatic types for two years before the current year were examined.

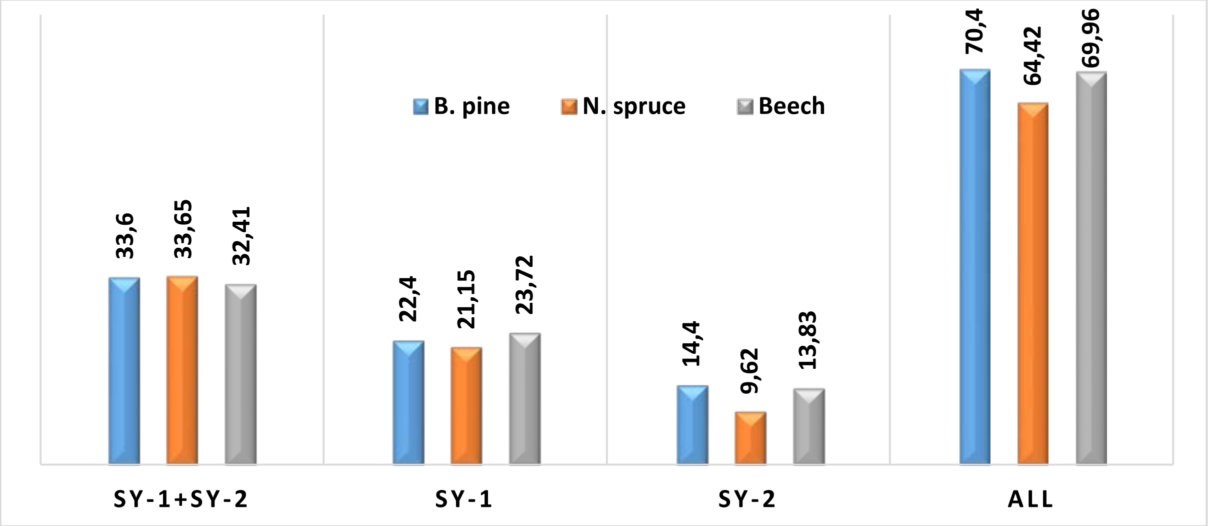

Fig. 4. *Percent participation of eustress years in preceding period of two years*

The pattern analysis show that the presence of eustress in the previous two years in 64 % to 70 % of the cases for the studied species has provoked eustres in current year. This in turn is related to the maintenance of one or both climatic regimes outside the climatic norms or the sudden changes in these regimes (Fig. 4). The cases, where eustres has existed in both previous years are more (32-34%), following the cases in which the previous year was eustress year (21-24%) and finally - when there was eustress two years ago (10-14 %).

Conclusion

For the studied mountain tree species and locations the original approach for meta-analysis, software, characteristics, indixes and coefficients were applied. The obtained eustress periods are maximal in number for the Bosnian pine, followed by beech. The average Depth, A_{av} = 0.16, 0.26 and 0.28; average Duration, D_{av} = 2, 2.5 and 2.3; and average Frequency, F_{av} = 10, 33 and 34 years over 100 years were obtained. Through the combined evaluation of the characteristics of eustress, the risk is established only for two studied locations of spruce and three locations of beech. The predominance of cold-dry and hot-wet years in total for the Bosnian pine number of adverse climatic years (with eustress), as well as hot-dry and cold-wet years for spruce and beech was established.

Pattern analysis presented proved that the change in one of two regimes - warm to cold or dry to wet, also the impact of adverse climatic type in at least two consecutive years and / or a presence of eustress in at least one of the previous years of three-yeas periods sure provoked eustress in the current year. So that sometimes the current adverse year belongs to normal or not so adverse climatic type.

The demonstrated holistic analysis allows to study and compare heterogeneous chronologies from different locations, differing in coverage, number of sequences, etc. These analysis would enable for better utilization of the existing international databases for assessing the adverse periods in the growth of the trees. Proposed holistic approach for meta-analyses of series (eustress identification in radial growth of stems) is convenient for the fast monitoring of forest communities state and for recognizing the stands under risk, where the eustress frequently appears, with very long duration and very deep depth. The capability of approach allows expression of reactive functional types of trees (PFTs) as groups of trees with the similar behavior to the influence of climatic types of years, and with similar characteristics of eustress periods.

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Manure, Soil Organic Matter and Soil Fertility

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Abstract

Higher content of soil organic matter can greatly reduce the difficulties of a good crop production system. Content of organic matter in the majority of Bulgarian soils is very low which is evident from the results of soil survey and the data from mass chemical soil tests – nearly 60% of studied soils are with humus content lower than 2.5%. The organic fertilization has direct effect on supplying humic substances in soil. On the basis of some Bulgarian soil types, it is shown the inclusion of organic fertilizers into the fraction of humic acids. A pot experiment and a small field study with Haplic Chernozems show an increase into the fraction of free humic acids and those bound with monovalent cations and mobile R_2O_3 oxides.

Key words: soil organic matter, nitrogen, fertilization, humic acids, Haplic Chernozems

Introduction

Humus content of the main soil types in Bulgaria is low (Angelov, E. et all, 1975, Filipov, H. 2003, Filcheva, E., Sl. Krastanov, 2003) compared to soils in the North of our country. This is due to a higher mineralization rate of crop residues as a result of a milder climate in Bulgaria. The actual problem in contemporary agriculture is the development of practices which will not only keep but also improve quantity and quality of soil organic matter. The question of quantity of organic substances which is needed to be added to soil in order to renew the loss of humus is important from scientific and practical aspect. It is known that organic matter decreases in consequence of the nutrient uptake by plants. The loss of soil organic matter has to be compensated, because it plays a key role in the limitation of nitrogen oxide emissions in the atmosphere.

The main task of this study is to establish the rate of humification of fresh manure (C: N=20.2) in Haplic Chernozem in dependence of applied fertilization rate which will serve as a base of evaluation of manure effectiveness on the accumulation of humus in soil. The other aim is to make an observation of soil types with low humus content and suggest good practices for improvement of agrochemical status of cultivated soils in Bulgaria on the basis of humus content of the main soil types, obtained at the agrochemical services in N. Poushkarov Institute of Soil Science, Agrotechnology and Plant Protection.

Materials and Methods

The studies are conducted as micro field experiment in Gorni Dabnik on Haplic Chernozem characterized with 0.13% total N and 2.4% humus. The added fresh no-straw-manure is labelled with ^{15}N isotope and contains 2.06% total N, 0.068% ammonium N and 0.024% nitrate N, C:N=20.2. The test crop is maize, (Pioneer 37-37), cultivated for grain. There are 3 treatments in 3 replications with plants and 1 replication with no plants. Each replication contains 41 kg soil. At the beginning and at the end of the experiment the quantity of total organic C was determined according to Tjurin's method

(Arinushkina, 1961) and the fractional composition of humus according to Kononova-Belchikova (Kononova, 1963).

Table 1. *Scheme of the micro field experiment*

Treatments	Replications			
	With plants			No plants
1-control-soil	1	2	3	4
2- soil+860g manure	1	2	3	4
3 soil+1720g manure	1	2	3	4

Results and discussion

The influence of manure (C: N=20.2), applied at rate 3 and 6 t/da on the total amount of C and the fractional composition of humus is presented in Table 2. At the beginning (application of manure) the increase of total organic C is significant, from 1.58 in the control soil to 1.69% in treatment with high rate of manure (GD=0.06). The change of C in humic acids is at the limit of the least significant difference. Therefore, we cannot affirmatively conclude that this increase is as an account of manure added organic material. A significant increase of C in humic acids was established 3 months later for the control soil Cx=0.37; for the treatment with the low rate of manure Cx=0.50 and for the one with the high rate Cx=0.56 (GD=0.08). The data from isotope analyses of N in humic acids confirm this assumption. While at the beginning of the experiment only 9.5- 8.5 % from added manure N is in humic acid fraction in pyrophosphate extract, 3 months later these values increase to 16-11.8% respectively for both the treatments with low and high rate of manure fertilization. The part of manure N in humic acid in alkaline extract after 3 months is lower – 12 and 8.2%. The quantity of fulvic acids weekly decrease for the mentioned period. Approximately by 6-7% also decreases the quantity of non-hydroxylated residue fraction in fertilized with manure treatments.

Table 2. *Fractional composition of humus after manure fertilization in micro field experiment*

Treatment	Days	Humus, %	Total C, %	C in pirophos. extract	Ch, %	Cf, %	Ch:Cf	From NaOH extract		C in residue	Cf in 0.1n H_2SO_4
								Ch free and bound R_2O_3	Ch bound with Ca		
1	**0**	2.72	1.58	0.56	0.39	0.17	2.3	0.07	0.32	1.02	0.04
1				35.4	24.7	10.7		17.9	82.1	64.1	2.5
				0.53	0.37	0.16		0.05	0.32	1.03	0.04
	90	2.69	1.56	34.0	23.7	10.3	2.3	13.5	86.5	66.0	2.6
	0	2.79	1.62	0.62	0.44	0.18	2.4	0.08	0.36	1.00	0.04
2				38.3	27.2	11.1		18.2	81.8	61.7	2.5
				0.63	0.50	0.13		0.08	0.42	0.97	0.05
	90	2.75	1.60	39.4	31.3	8.1	3.8	16.0	84.0	60.6	3.1
	0	2.91	1.69	0.63	0.44	0.19	2.3	0.05	0.39	1.06	0.04
3				37.3	26.0	11.3		11.4	88.6	62.7	2.5
				0.69	0.56	0.13		0.11	0.45	1.00	0.05
	90	2.91	1.69	40.8	33.1	7.7	4.3	19.6	80.4	59.2	3.0

* GD 0 0.06 0.05
GD 90 004 0.08

The mentioned increase in humic acids in fertilized plots without plants is observed in the relevant treatments with plants at the end of the experiment. The results indicate a significant increase in the group of free and mobile R_2O_3 bound humic acids under the influence of plants. The changes vary from 0.08 (16%) and 0.11 (19.6%) in the treatments without plants to 0.11 (22%) and 0.17 (30.9%) in those with plants, respectively for treatments with low and high rate of fertilization. The quantity of hydrolysate residue decreases significantly which is due to plant vegetation. This is a proof that plant growth increases organic matter mineralization rate. Over time, the carbon in the fraction of free and R_2O_3 oxides bound humic acids increases. Such a tendency was established also in the pot experiment with Haplic Chernozem (Petkova, 2001).

The different conditions of soil formation in Bulgaria determine the formation of soils with different fertility and agricultural properties presented in 15 soil types. In soil-agrochemical studies are included 11 groups of soils, of which the most prevalent are Cinnamonic forest soils (29% of the country's territory), followed by the Chernozem soils (29% of the country's territory), the Grey forest soils, 17%, Brown forest, 15%, Aluvial-meadows soils, 7%, Vertisols, (Smolnica), 6% of the area of the country.

All the cultivated land accounts to ~ 52% of area of the country. These cultivated lands are the so called deep soils. They are characterized with significant fertility and favourable agrotechnical properties, and occupy the plains and hilly terrains, where are possible full mechanization and modern artificial irrigation and fertilization. In recent years economic activity has an adverse effect on soil resources in three directions: reduction of arable land, increasing the degraded soils (eroded, surface waterlogged, acidic and saline) and contamination of soils. Climate changes create significant problems, which increase the mineralization of soil organic matter and thus contribute to the reduction of humus in the soil.

The analysis of the results of soil-agrochemical studies on the content of humus in the main soil types presented in Table 3 indicates that the highest is the percentage of soils with humus content between 1-2.5%- totally 52.23% of the cultivated land. Approximately 30% of soils fall among the next column of the Table with 2.5- 3.5% humus content. In accordance with the Grishina's (Grishina, 1986) gradation scale these soils are with very low (2%) < and low content of humus (2-4%). It is known that humus content defines the potential fertility and correlates with the stock of basic nutrient elements. The quantity of total nitrogen also changes in most of the cases according to humus content in soil (Fokin, 1986, Stevenson, 1982, Mooleki, et al. 2004). It is therefore, necessary to take measures to maintain and increase the content of soil organic matter which will lead to improvement of soil fertility. The root and post-harvest residues are the main resources for adding to organic matter in soil. There is a serious threat to strong disturbance of the balance of soil organic matter in nowadays tendency of decreasing organic fertilization. The addition of farmyard manure and other organic materials together with leaves, stems and roots from the residual products of cultivated agricultural crops creates favorable conditions for formation of young organic matter. Their faster mineralization provides effective nutrient regime for crop vegetation. Nutrient management is a key factor in organic agriculture. For some nutrients, a balance approach is an option, however for nitrogen this is not sufficient enough. Availability and crop demand should be synchronized as close as possible for optimal efficiency and minimal losses. Because of the number and complexity of processes involved, a model approach can be useful (Burgt et al., 2009).

Table 3. *Analyses of the results from soil agrochemical tests in Bulgaria*

	up to 1%	Humus content in A horizon					from3,5 to 5%		over5%		total	avarage
	1%		1% - 2,5%		2,5 - 3,5%		3,5 - 5%		5%			
Groups Soils	Number of fields	da	Number of fields	da	Number of fields	da	Number of fields	da	Number of fields	da	da	In groups
1	57	22818	3531	2046128	3449	2431121	1178	875063	34	19162	5394292	2.78
2	79	42276	4906	3094648	4124	3014865	786	536756	34	30973	6719518	2.6
3	35	17787	4277	2446557	1248	693352	140	74567	11	5466	3237729	2.15
4	329	84162	5055	1897689	649	259019	248	69376	25	11335	2321581	1.98
5	55	14048	713	277253	104	34883	39	9246	19	2259	337689	1.94
6	48	13981	3056	1280685	3514	1682879	1168	631713	60	30106	3639364	2.84
7	3940	1118224	8009	2724352	994	360298	444	150556	76	26542	4379072	1.65
8	393	124754	560	186859	14	6462	0	0	8	2640	320715	1.24
9	1198	471203	1260	275370	206	30932	131	22125	92	25407	825037	1.56
10	1824	638396	7553	2444701	1921	607108	844	286098	367	91102	4067405	2.03
11	87	22620	505	130934	367	104769	115	29499	126	40279	328101	2.98
Sum	8045	2570269	39425	16805176	16590	9225688	5093	2864999	852	285271	31571403	
%		8.14%		53.23%		29.22%		8..5%		0.9%		

Legend:

1. Calcic Chernozems
2. Haplic Chernozem
3. Luvic Chernozems, Luvic Phaeozems
4. Luvisols
5. Planosols
6. Vertisols and Pfaeozems
7. Luvisols

8 Planosols

9. Cambisols, Umric Cambisols, Umrisols
10. Fluvisols, Gleyic Luvisols, Gleyic Chernozems, Gleyic sols, Saline soils
11. Rendzic Leptosols

^{x} – according to WRBSR (2006)

Conclusion

The isotopic analysis of nitrogen in the fraction of humic acids indicates that three months (90 days) after the introduction of manure with C:N = 20.2, the amount of carbon in humic acids increases from 0,37 in the control soil to 0.50% in the treatments fertilized with low rate of manure and to 0.56% (GD=0.08), respectively in those fertilized with high rate of manure. While at the beginning of the experiment only ~ 9.5 - 8.5% of the manure added nitrogen is in the fraction of humic acids, in the pyrophosphate extract three months later these values increased to 16 and 11.8%, respectively for treatments with low and high rate of fertilization. The contribution of fertilizer nitrogen in the fraction of humic acids in the alkaline extract after 3 months was low, 12 and 8.2%. The amount of fulvic acids after 90 days of experiment slightly decreases. By 6-7% decreases the amount of non-hydrolysable residue in treatments fertilized with manure. The introduction of organic manure contributes not only to an increase in humus content, but also to improvement of its quality by increasing the carbon stock in the fraction of humic acids. A significant increase into the fraction of free humic acids and those bound with monovalent cations and mobile R_2O_3 oxides under the influence of plants was registered. From 0.08 (16%) and 0.11 (19.6%) in the treatments without plants the humic acids contents change to 0.11 (22%) и 0.17 (30.9%) in the treatments with plants.

The analysis of the results of soil-agrochemical studies on the content of humus in the main soil types indicates that the highest is the percentage of soils with humus content between 1-2.5%- presented as 52.23% from cultivated lands. Approximately 30% of soils are with 2.5-3.5% humus content which characterizes them as soils with very low (<2%) and low (2-4%) content of humus. This requires to focus farmer’s attention on the opportunities for efficient utilization of all natural resources, farmyard manure, poultry manure, as well as organic residues such as leaves, stems and roots of the residual production of crops and various composts of organic material, in order to replenish stocks of humus in soil. Periodic soil application of nitrogen-containing organic materials alongside with mineral fertilization ensures the preservation of the natural composition of nitrogen in soil organic matter and its mobility. This will provide conditions for a positive balance of soil organic matter by keeping a balance between the processes of mineralization and humification. This efficient agricultural activity will lead to sustainable use of soil and will provide stable and high quality yields.

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Some examples for references to journal publications, books, chapters in edited books and electronic publications or databases:

- Atanassova, I., S. Doerr, 2010. Organic compounds of different extractability in total solvent extractsfrom soils of contrasting water repellency. European Journal of Soil Science, 61, 298-313.
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- Noyes, J. S. 2013. Universal Chalcidoidea Database. World Wide Web electronic publication. Last update 29 August 2013. URL: http://www.nhm.ac.uk/chalcidoids.

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